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Carolanne M. King
Carolanne M. King



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty. Dkt. No.: 4100.000582

Prior Application Serial No.:
08/479,722Prior Application Examiner:
David L. Fitzgerald

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

Prior Group Art Unit: 1646

**REQUEST FOR FILING CONTINUATION
APPLICATION UNDER 37 C.F.R. § 1.53(b)**

This is a request for filing a continuation application under Rule 53(b) (37 C.F.R. § 1.53(b)) of co-pending prior application Serial No. 08/479,722 filed June 7, 1995, entitled "Latent TGF β Binding Protein (LTBP) Genes, Compositions and Methods", now amended to read "Recombinant Production of Latent TGF β Binding Protein-3 (LTBP-3)", now entitled "Methods of Using Latent TGF β Binding Proteins".

- ☒ 1. Enclosed is a copy of the prior application Serial No. 08/479,722 as originally filed, including specification, claims, drawings, and declarations (properly submitted after the filing date). The undersigned hereby verifies that the attached

papers are a true copy of the prior application as originally filed and identified above, that no amendments (if any) referred to in the declaration filed to complete the prior application introduced new matter therein, and further that this statement was made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statement may jeopardize the validity of the application or any patent issuing thereon.

(a) ☒ The inventorship is the same as prior Application Serial No. 08/479,722.

(b) ☐ Deletion of inventor(s). Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. § 1.63(d)(2) and 1.33(b).

☒ 2. Enclosed is a check in the amount of \$654.00 to cover the filing fee as calculated below and the fee for any new claims added in the Preliminary Amendment referred to in Clause No. 7 below.

CLAIMS AS FILED IN THE PRIOR APPLICATION
LESS CLAIMS CANCELED BELOW

FOR	NUMBER FILED	NUMBER EXTRA	RATE	FEE
Basic Fee -----				\$345.00
Total Claims	37 - 20 =	17 X	\$ 9.00 =	\$153.00
Independent Claims	7 - 3 =	4 X	\$39.00 =	\$156.00
Multiple Dependent Claim(s) -----				\$0.00
TOTAL FILING FEES:				\$654.00



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- 002799 582560
- ☒ 3. Applicant is entitled to Small Entity Status for this application.
- ☐ (a) A small entity statement is enclosed.
- ☒ (b) A small entity statement was filed in the prior nonprovisional application and such status is still proper and desired (copy attached).
- ☐ (c) Small entity status is no longer claimed.
- ☒ 4. If the check is missing or insufficient, the Assistant Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16 to 1.21 which may be required for any reason relating to this document, or credit any overpayment to Williams, Morgan & Amerson, P.C. Deposit Account No. 50-0786/4100.000582.
- ☒ 5. Enclosed is a copy of the former and current Power of Attorney in the prior application.
- ☒ 6. Address all future communications to:
- Shelley P.M. Fussey
WILLIAMS, MORGAN & AMERSON, P.C.
7676 Hillmont, Suite 250
Houston, Texas 77040
(713) 934-7000
- ☒ 7. The prior application is presently assigned to The Regents of The University of Michigan (copy of Assignment attached).
- ☒ 8. Enclosed is a preliminary amendment (including Exhibits A through F). Any additional fees incurred by this amendment are included in the check at No. 2 above and said fee has been calculated after calculation of claims and after amendment of claims by the preliminary amendment.

- 002190 58926550
- ☒ 9. Cancel in this application claims 1-39, 41 and 42 (*i.e.*, all claims except claim 40) of the prior application before calculating the filing fee. (At least one original independent claim must be retained).
- ☐ 10. Amend the specification by inserting before the first line the sentence: --This is a continuation of co-pending application Serial No. _____ filed _____.
- ☒ 11. Enclosed are formal drawings of FIG. 1 through FIG. 8C on a total of 8 sheets.
- ☒ 12. The present paper constitutes Applicants' compliance with their duty of disclosure under 37 C.F.R. § 1.56. Please make all prior art of record in parent application Serial No. 08/479,722 of record in this case. As a courtesy, the following is enclosed:
- ☒ (a) Copies of PTO-1449s from prior application.
- ☐ (b) Copies of IDS citations.
- ☒ 13. Transfer the sequence information, including the computer readable form submitted on August 24, 1999 in the parent application, Serial No. 08/479,722, for use in the application. 37 C.F.R. § 1.821(e). As a courtesy, a paper copy is enclosed.
- ☒ 14. Return Receipt Postcard.

Respectfully submitted,



Shelley P.M. Fussey
Reg. No. 39,458
Patent Agent

WILLIAMS, MORGAN & AMERSON
7676 Hillmont, Suite 250
Houston, Texas 77040
(713) 934-7000
Date: June 12, 2000



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Jeffrey Bonadio and
Wushan Yin
Serial No.: 08/479,722
Filed: June 7, 1995
For: LATENT TGFB BINDING
PROTEIN (LTBP) GENES,
COMPOSITIONS AND METHODS

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Group Art Unit: Unknown
Examiner: Unknown
Atty. Dkt.: UMIC:013/FUS

DECLARATION CLAIMING SMALL ENTITY STATUS
37 C.F.R. §§ 1.9(f) and 1.27(d) - NONPROFIT ORGANIZATION

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

Name of Organization: The Regent of the University of Michigan

Address of Organization: 3003 South State Street
Wolverine Tower, Room 2071
Ann Arbor, Michigan 48109-1280

The type of organization is a university.

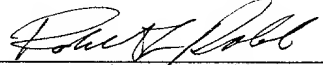
I hereby declare that the organization identified above qualifies as a nonprofit organization as defined in 37 C.F.R. § 1.9(e) (1), and thus is a "small entity" as defined in § 1.9(f), for purposes of paying reduced fees under Sections 41(a) and (b) of Title 35, United States Code, with regard to the above-referenced application.

I hereby declare that exclusive rights to the invention have been conveyed to and remain with the organization, with respect to the above-referenced invention, nor have I assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR § 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR § 1.9(d) or a nonprofit organization under 37 CFR § 1.9(e), with the exception that the Government may have rights in the invention pursuant to a funding agreement under 35 U.S.C. § 202(c)(4): NIH Grant No. HL-41926.

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate.

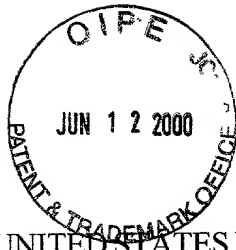
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

THE REGENT OF THE UNIVERSITY OF MICHIGAN

By: 
Name: Robert L. Robb
Title: Director of Technology
Management Office

Date: 9-13-95

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Jeffrey Bonadio & Wushan Yin

Serial No.: Unknown
Parent Serial No.: 08/479,722

Filed: June 12, 2000
Parent Filed: June 07, 1995

For: METHODS OF USING
LATENT TGF β BINDING
PROTEINS

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§ Group Art Unit: 1646
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§ Examiner: Fitzgerald, D. L.
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§ Atty. Dkt.: 4100.000582
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<p>"EXPRESS MAIL" MAILING LABEL</p> <p>NUMBER EL 332 810 365 US DATE OF DEPOSIT: June 12, 2000</p> <p>I hereby certify that this paper or fee is being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service on the date indicated above and is addressed to Assistant Commissioner for Patents, Washington, D.C. 20231</p> <p><i>Carolanne M. King</i> Carolanne M. King</p>

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination, the Examiner is respectfully requested to enter the following amendments. Remarks supporting patentability of all claims are also included, which the Examiner is respectfully requested to consider. In light of the issuance or allowance of the three earlier applications to which priority is claimed, examination and consideration with a view to a timely allowance is respectfully requested.

AMENDMENT

In the Specification:

Please delete the title from the parent application and replace with the new title -- METHODS OF USING LATENT TGF β BINDING PROTEINS --.

At page 2, line 5, after "30, 1994", please insert --, which issued as U.S. Patent No. 5,942,496 on August 24, 1999 --.

At page 2, line 6, after "18, 1994", please insert --, which issued as U.S. Patent No. 5,763,416 on June 09, 1998 --.

At page 21, beginning at line 24 and extending through page 22, line 15, please delete the entire text beginning with "**FIG. 9.**" and extending through "40091 antiserum."

At page 48, line 9, after the paragraph that ends with "manner.", please insert the following new paragraph -- Accordingly, an LTBP protein or polypeptide may be provided to a repair tissue site or bone progenitor tissue site. A nucleic acid segment (DNA or RNA) that expresses an LTBP protein or polypeptide in cells of the tissue site may be provided, as may a nucleic acid segment in association with a structural biocompatible matrix (U.S. Patent No. 5,942,496 and U.S. Patent No. 5,763,416). --.

At page 75, line 7, after "pLTBP-3fl" please insert --, deposited November 24, 1997 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 (now at 10801 University Boulevard, Manassas, VA 20110-2209), and given the ATCC Accession numbers ATCC 209496 --.

At page 75, line 11, after "1995)", please delete "(FIG. 9)".

At page 75, at blank line 12, please insert -- Co-transfection of 293T cells with pLTBP-3fl and pTGF- β 1 was followed by immunoprecipitation of LTBP-3 and TGF- β 1 produced by

293T cells following transient transfection and radiolabeling. Aliquots ($\sim 10^6$ incorporated CPM) of radiolabeled media were immunoprecipitated and separated using 4%-18% gradient SDS-PAGE and either reducing or nonreducing conditions as described (Yin et al., 1995). Cold standards were used to estimate molecular weights (200, 97.4, 69, 46, 30, 21.5 and 14.3 kDa; Rainbow mix, Amersham). The immunoprecipitation was followed by: 1, 293T cell medium proteins immunoprecipitated with 274 antibody (separated under reducing conditions) after co-transfection with pLTBP-3fl and pTGF- β 1; 2, 293T cell medium proteins immunoprecipitated with 274 antibody (separated under nonreducing conditions) after co-transfection with pLTBP-3fl and pTGF- β 1; 3, untransfected 293T cell medium proteins immunoprecipitated with 274 antibody (separated under reducing conditions); 4, 293T cell medium proteins immunoprecipitated with 274 antibody (separated under reducing conditions) after transfection with pLTBP-3fl; 5, 293T cell medium proteins immunoprecipitated with 40091 antibody (separated under reducing conditions) after transfection with pTGF- β 1; 6, 293T cell medium proteins immunoprecipitated with 40091 antibody (separated under nonreducing conditions) after transfection with pTGF- β 1; and Lane 7, 293T cell medium proteins immunoprecipitated with 40091 antibody (separated under reducing conditions) after co-transfection with pLTBP-3fl and pTGF- β 1. The signal was weakest in lanes in which proteins were immunoprecipitated using the 40091 antibody, reflecting the weaker affinity of the 40091 antiserum. --.

At pages 92-118, please delete the entire sequence listing. Please re-number the following pages (claims and abstract) consecutively.

After page 127 (abstract), please insert the correct sequence listing pages submitted August 24, 1999 in the parent application (see accompanying Request at Box 13) and number these pages separately beginning with page 1.

In the Claims:

The accompanying paper requests cancellation of all pending claims except claim 40, without prejudice or disclaimer.

Please amend claim 40 as follows:

40. (Amended) A method for [identifying] binding a transforming growth factor β (TGF- β) protein in a sample, comprising contacting said sample with [an] a purified mammalian LTBP-2 or LTBP-3 protein or polypeptide under conditions effective to allow binding [and detecting the protein so bound] of said LTBP-2 or LTBP-3 protein or polypeptide to said TGF- β protein; wherein said LTBP-2 or LTBP-3 protein or polypeptide comprises at least fifteen contiguous amino acids present in SEQ ID NO:2 or SEQ ID NO:4, respectively.

Please add new claims 43-78, as follows:

--

43. The method of claim 40, wherein said sample is located within an animal and said LTBP-2 or LTBP-3 protein or polypeptide is administered to said animal in an amount effective to bind TGF- β in said animal.

44. A method of binding TGF- β , comprising contacting a composition comprising TGF- β with a composition comprising a purified mammalian LTBP-2 or LTBP-3 protein or polypeptide in an amount effective to bind TGF- β ; wherein said LTBP-2 or LTBP-3 protein or polypeptide comprises at least fifteen contiguous amino acids present in SEQ ID NO:2 or SEQ ID NO:4, respectively.

45. The method of claim 44, wherein said composition comprising TGF- β is located within an animal and said composition comprising said LTBP-2 or LTBP-3 protein or polypeptide is administered to said animal in an amount effective to bind TGF- β in said animal.

46. A method of using an LTBP-2 or LTBP-3 protein, polypeptide or peptide, comprising providing to an animal a biologically effective amount of a purified mammalian LTBP-2 or LTBP-3 protein, polypeptide or peptide that comprises at least fifteen contiguous amino acids present in SEQ ID NO:2 or SEQ ID NO:4, respectively.

47. The method of claim 46, wherein an amount of an LTBP-2 or LTBP-3 protein, polypeptide or peptide effective to generate anti-LTBP-2 or anti-LTBP-3 antibodies is provided to said animal.

48. The method of claim 47, wherein an LTBP-2 or LTBP-3 peptide of between 15 and about 50 amino acids in length is provided to said animal.

49. The method of claim 47, wherein an LTBP-2 or LTBP-3 peptide of between 15 and about 30 amino acids in length is provided to said animal.

50. The method of claim 47, wherein antisera comprising said anti-LTBP-2 or anti-LTBP-3 antibodies is collected from said animal.

51. The method of claim 46, wherein an amount of an LTBP-2 or LTBP-3 protein or polypeptide effective to bind TGF- β is provided to said animal.

52. The method of claim 51, wherein LTBP-2 or LTBP-3 binding to TGF- β regulates TGF- β activity in said animal.

53. The method of claim 51, wherein LTBP-2 or LTBP-3 binding to TGF- β modulates the activation of TGF- β in said animal.

54. The method of claim 51, wherein LTBP-2 or LTBP-3 binding to TGF- β modulates the activation of latent complexes that comprise TGF- β , thereby regulating TGF- β activity.

55. The method of claim 51, wherein LTBP-2 or LTBP-3 binding to TGF- β targets TGF- β to the extracellular matrix in said animal.

56. The method of claim 51, wherein LTBP-2 or LTBP-3 binding to TGF- β targets TGF- β to the bone matrix in said animal.

57. The method of claim 51, wherein LTBP-2 or LTBP-3 binding to TGF- β targets TGF- β to connective tissues in said animal.

58. The method of claim 51, wherein LTBP-2 or LTBP-3 binding to TGF- β targets TGF- β to the cell surface of cells in said animal.

59. The method of claim 51, wherein LTBP-2 or LTBP-3 binding to TGF- β protects TGF- β from proteolytic attack and activation in said animal.

60. The method of claim 51, wherein LTBP-2 or LTBP-3 binding to TGF- β protects TGF- β from proteolytic attack and activation during wound repair or tissue healing in said animal.

61. The method of claim 51, wherein said LTBP-2 or LTBP-3 protein or polypeptide is a recombinant protein or polypeptide prepared by expressing an LTBP-2-encoding or LTBP-3-encoding DNA segment in a recombinant host cell and purifying the expressed LTBP-2 or LTBP-3 protein or polypeptide away from total recombinant host cell components.

62. The method of claim 51, wherein said TGF- β is located within a tissue healing, wound repair tissue site or bone progenitor tissue site of said animal and wherein said LTBP-2 or LTBP-3 protein or polypeptide is provided to said tissue site.

63. The method of claim 62, wherein said TGF- β is located within a tissue healing or wound repair tissue site of said animal.

64. The method of claim 62, wherein said TGF- β is located within a bone progenitor tissue site of said animal.

65. The method of claim 62, wherein said LTBP-2 or LTBP-3 protein or polypeptide is provided to said tissue site by contacting said tissue site with a composition comprising a nucleic acid segment that expresses said LTBP-2 or LTBP-3 protein or polypeptide in cells of said tissue site.

66. The method of claim 65, wherein said LTBP-2 or LTBP-3 protein or polypeptide is provided to said tissue site by contacting said tissue site with a composition comprising said nucleic acid segment and a structural biocompatible matrix.

67. The method of claim 65, wherein said nucleic acid segment is a DNA segment.

68. The method of claim 65, wherein said nucleic acid segment is an RNA segment.

69. The method of claim 51, wherein said LTBP-2 or LTBP-3 protein or polypeptide comprises at least about thirty contiguous amino acids present in SEQ ID NO:2 or SEQ ID NO:4, respectively.

70. The method of claim 51, wherein said LTBP-2 or LTBP-3 protein or polypeptide comprises at least about fifty contiguous amino acids present in SEQ ID NO:2 or SEQ ID NO:4, respectively.

71. The method of claim 51, wherein said LTBP-2 or LTBP-3 protein or polypeptide exhibits at least 90% identity to the amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4, respectively.

72. The method of claim 51, wherein said LTBP-2 or LTBP-3 protein or polypeptide exhibits between 91% and about 99% identity to the amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4, respectively.

73. The method of claim 51, wherein an LTBP-2 protein comprising the amino acid sequence of SEQ ID NO:2 is provided to said animal.

74. The method of claim 51, wherein an LTBP-3 protein comprising the amino acid sequence of SEQ ID NO:4 is provided to said animal.

75. A method of using an LTBP-2 or LTBP-3 protein or polypeptide, comprising administering to an animal a purified mammalian LTBP-2 or LTBP-3 protein or polypeptide in an amount effective to bind TGF- β in said animal; wherein said LTBP-2 or LTBP-3 protein or polypeptide specifically binds TGF- β and comprises at least fifteen contiguous amino acids present in SEQ ID NO:2 or SEQ ID NO:4, respectively.

76. A method of binding TGF- β within a repair or bone progenitor tissue site of an animal, comprising contacting said tissue site with a purified mammalian LTBP-2 or LTBP-3 protein or polypeptide, or a nucleic acid that expresses said LTBP-2 or LTBP-3 protein or polypeptide, to

provide an amount of said LTBP-2 or LTBP-3 protein or polypeptide effective to bind TGF- β in said animal; wherein said LTBP-2 or LTBP-3 protein or polypeptide comprises at least fifteen contiguous amino acids present in SEQ ID NO:2 or SEQ ID NO:4, respectively.

77. A method of binding TGF- β , comprising administering to an animal a composition comprising a purified mammalian LTBP-2 or LTBP-3 protein or polypeptide in an amount effective to bind TGF- β in said animal; wherein said LTBP-2 or LTBP-3 protein or polypeptide comprises at least fifteen contiguous amino acids present in SEQ ID NO:2 or SEQ ID NO:4, respectively.

78. A method of binding TGF- β , comprising administering to an animal a composition comprising a purified mammalian LTBP-3 protein or polypeptide in an amount effective to bind TGF- β in said animal; wherein said LTBP-3 protein or polypeptide binds TGF- β and comprises at least fifteen contiguous amino acids present in SEQ ID NO:4 or exhibits at least 90% identity to the amino acid sequence set forth in SEQ ID NO:4. --.

REMARKS

I. Status of the Claims

The accompanying paper requests cancellation of all pending claims except claim 40, without prejudice or disclaimer. Presently, claim 40 is being amended to even further improve its clarity. New claims 43-78 have been added, which are supported by the original specification and parent applications incorporated therein by reference (see below). Numbering the present

claims starting with claim 43 is believed to be correct as the copy of the specification enclosed concludes with claim 42.

Claims 40 and 43-783 are therefore in the case. For the convenience of the Examiner, a copy of the pending claims is attached hereto as **Exhibit A**.

II. Continuing Application Status

The present application is a continuing application based upon allowed application Serial No. 08/479,722 ("the '722 application; Attorney Docket Nos. 4100.000500, UMIC:013). The inventorship remains the same as the earlier application.

A copy of the claims that will issue from the '722 application is enclosed as **Exhibit B**. The claims canceled from the '722 application in light of a restriction requirement are also provided herewith as **Exhibit C**.

III. Other Parent Applications

The parent, '722 application is a continuation-in-part of PCT Application PCT/US95/02251, filed February 21, 1995 (Attorney Docket Nos. 4100.000410, UMIC:009P); which is a continuation-in-part of U.S. Application Serial Number 08/316,650 ("the '650 application; Attorney Docket Nos. 4100.000300, UMIC:003), filed September 30, 1994; which is a continuation-in-part of U.S. Application Serial Number 08/199,780 ("the '780 application; Attorney Docket Nos. 4100.000200, UMIC:002), filed February 18, 1994. The '650 application matured into U.S. Patent No. 5,942,496, and the '780 application earlier matured into U.S. Patent No. 5,763,416.

The entire text and figures of each of the '650 and '780 applications were specifically incorporated by reference into the '722 application on filing (see '722 application at page 2, opening paragraph).

A copy of the claims to issue from the '650 application is enclosed as **Exhibit D**. The claims that issued from the '780 application are provided as **Exhibit E**, and the original claims in the '780 application are shown in **Exhibit F**.

IV. Amendments to the Specification

As the grandparent ('650) and great grandparent ('780) applications were specifically incorporated by reference into the immediate parent ('722) application (see page 2), the text of the '650 and '780 applications forms part of the present disclosure. An amendment is presently being introduced into the instant specification from those earlier applications, to which priority is still claimed. This addition is fully supported by the original '650 and '780 applications and, for convenience of review, may be correlated with the issued claims in those cases attached as **Exhibit D** and **Exhibit E**.

Incorporation of material into the specification by reference is proper. *Ex parte Schwarze*, 151 USPQ 426 (Bd. App. 1966). Incorporation by reference of one or more issued U.S. patents, at the time of filing the application, also provides the enabling support required by 35 U.S.C. § 112, first paragraph, for any claims advanced in the new application that rely on the same standard of teaching of "how to make and use" as the claims in the issued patent(s).

Other issued U.S. patents that concern the use of exogenous growth factors in methods for treating bone defects or inducing bone formation *in vivo* are also incorporated by reference into the '722 specification. For example, U.S. Patent No. 4,877,864, which concerns the

administration of a therapeutic composition of bone inductive protein to treat cartilage and/or bone defects, and U.S. Patent 5,108,753, which concerns the use of a device containing a pure osteogenic protein to induce endochondral bone formation and for use in periodontal, dental or craniofacial reconstructive procedures ('722 specification at pages 30 and 83).

Upon agreement concerning allowable subject matter, Applicants intend to amend the 'Summary of Invention' section to add the precise language of the allowed claims. In the interest of efficiency for both the Applicants and the Office, it seems most appropriate to defer amendment of the Summary until agreement on allowability.

Certain amendments to the specification are also being made to conform the specification to the formal drawings enclosed herewith. These amendments, each of which were entered during prosecution of the '722 application, are fully supported by the original text and drawings.

V. Support for the Claims

Support for the present claims is to be found throughout the original parent ('722) application and in the grandparent ('650) and great grandparent ('780) applications, each specifically incorporated into the '722 application by reference and now progressed to issue.

In addition to finding support throughout the present specification, the language of the current claims matches that found to be acceptable in the parent applications, including the definitions of the proteins, polypeptides, peptides and nucleic acids from the '722 application and the tissue site and gene-matrix embodiments from the '650 and '780 applications. For the convenience of the Examiner, copies of the allowed claims from each of the three parent applications are attached hereto as **Exhibits B, D and E**. Additional support for the present claims exists in the specification and parent applications as follows.

Claim 40 is based upon the original claim (**Exhibit C**), although "identifying" has been replaced with "binding". Claim 44 is also based upon a version of claim 40 that includes the "binding" terminology. "Binding TGF β " is evidently an inherent property of latent TGF β binding proteins (LTBPs) and is supported by the entire '722 specification (*e.g.*, see page 47, line 14 and page 68, line 5).

The LTBP-2 and LTBP-3 proteins and polypeptides in all independent claims are defined as "purified, mammalian" proteins or polypeptides, as in the claims allowed in the parent application (**Exhibit B**).

In independent claims 40, 44, 46, 75, 76 and 77, the language comprising "at least fifteen contiguous amino acids present in SEQ ID NO:4", as allowed in the parent application in reference to LTBP-3 (**Exhibit B**), has also been adapted for LTBP-2. In independent claim 78, which refers to LTBP-3 alone, the protein or polypeptide is defined in the alternative as either comprising at least fifteen contiguous amino acids from SEQ ID NO:4 or exhibiting at least 90% identity to the amino acid sequence of SEQ ID NO:4. These definitions were allowed in claims 127 and 145 of the '722 application (**Exhibit B**).

Dependent claims 43 and 45 qualify independent claims 40 and 44 by reciting that the LTBP-2 or LTBP-3 proteins or polypeptides are administered to an animal to bind TGF- β . These claims are supported throughout the '722 application, *e.g.*, at least at page 29, line 10 through page 30, line 28; page 44, line 30 through page 48, line 8; page 57, line 17 through page 58, line 29; and at page 71, line 7 through page 74, line 2.

Similarly, independent claim 46 recites methods of use in which LTBP-2 or LTBP-3 proteins, polypeptides or peptides are administered to an animal, and is supported by the '722 application at least at page 2, lines 12-14; page 29, line 10 through page 30, line 28, as

exemplified by page 29, line 11 and page 30, lines 22-28; page 41, line 19 through page 42, line 4; page 44, line 30 through page 48, line 8; page 57, line 17 through page 58, line 29; page 71, line 7 through page 74, line 2; and at page 127, line 4.

Claims 47 and 50 concern the administration of LTBP-2 or LTBP-3 proteins, polypeptides or peptides to generate anti-LTBP-2 or anti-LTBP-3 antibodies and antisera that may be collected from an animal. These are supported by Section 8 of the Detailed Description and in Example II of the '722 application, see, *e.g.*, page 40, lines 25-27. The exemplary peptides of between 15 and about 50 or 30 amino acids in length, as defined in claims 48 and 49, are supported by allowed claims 130 and 131 in the '722 application (**Exhibit B**).

Dependent claims 51 through 60 recite various functional outcomes of LTBP binding to TGF- β , and are supported throughout the '722 application, notably by Sections 9 and 10 of the Detailed Description and in Example I. Basically, as TGF- β has the ability to regulate tissue remodeling and wound repair (see pages 2-8, 46, 47 and 57), and as LTBPs bind TGF- β (Example I), LTBPs are useful in the regulation of tissue remodeling and wound repair.

The production and storage of TGF- β as a latent complex that is activated only under certain physiological (or pathological) conditions provides for the precise regulation of TGF- β (pages 4, 6, 8, 47, 57, 71 and 73). LTBP_s both regulate and target TGF- β activity (pages 45 and 71) and help ensure TGF- β integrity *in vivo* (page 46). By protecting TGF- β from proteolytic attack under pathological conditions, LTBP_s will moderate aberrant effects, such as reducing scar tissue during wound healing. LTBP_s thus contribute to TGF- β regulation through a sophisticated feedback loop (pages 71 and 73).

For particular written description support for claims 52 through 60, see page 45, line 23 (claim 52; regulates TGF- β activity); page 46, line 5 and page 58, lines 22-27 (claims 53 and 54;

modulates the activation of latent complexes that comprise TGF- β); page 46, line 1 and page 58, lines 19-22 (claim 55; targets TGF- β to the extracellular matrix); page 48, lines 4-5 (claim 56; targets TGF- β to the bone matrix); page 45, line 29 and page 58, line 18 (claim 57; targets TGF- β to connective tissues); page 46, line 2 and page 58, lines 27-29 (claim 58; targets TGF- β to the cell surface); and page 3, lines 14, 15, 26 and 27; page 4, line 14; page 6, line 29; page 28, line 9; page 46, lines 7-11 and page 58, lines 27-29 (claims 59 and 60; protects TGF- β from proteolytic attack and activation, as in wound repair and tissue healing).

Claim 61 defines the LTBP-2 or LTBP-3 proteins or polypeptides as recombinant proteins or polypeptides and is based upon original claim 22 (**Exhibit C**).

Each of claims 62-64 define the location of the TGF- β within the animal as a tissue healing, wound repair tissue site or bone progenitor tissue site. These are again supported throughout the '722 application, notably by Sections 1-4, 9 and 10 of the Detailed Description and Example I (see also pages 2-8, 22, 27, 28, 46, 47, 57 and 58, particularly page 3, lines 26-27; page 4, line 14; page 6, line 29; page 28, line 9; page 46, lines 8-11; page 47, lines 19-22; and page 48, line 1). The "repair" and "bone progenitor" tissue sites also correlate with the language of the gene-matrix method claims allowed in the grandparent ('650) and great grandparent ('780) applications (**Exhibit D** and **Exhibit E**).

Claims 65-68 concern the use of nucleic acids (claim 65) and nucleic acid-structural matrix compositions (claim 66) to provide the LTBP-2 or LTBP-3 protein or polypeptide to TGF- β in a repair or bone progenitor tissue site of an animal. Both DNA (claim 67) and RNA (claim 68) nucleic acids may be employed (see also claims 161 and 162 of the '722 application, **Exhibit B**). RNA may be preferred in certain embodiments, particularly as traversal of the nuclear envelope is not necessary for expression.

The use of nucleic acids and nucleic acids in functional association with structural matrices are predominantly supported by the '650 and '780 applications specifically incorporated by reference into the '722 application (see **Exhibits D, E and F**). Accordingly, such language is being introduced into the present application by amendment at a convenient point of the detailed description. Incorporation of material into the specification by reference is proper. *Ex parte Schwarze, supra*.

Exemplary LTBP-2 and LTBP-3 proteins and polypeptides for use in the claimed invention are described in claims 69 through 74. These claims are based upon those allowed in the '722 application (see claims 128, 129, 138 and 144-146 of **Exhibit B**).

Claims 75 through 78 are variations of earlier claims presented in independent form. Support for these claims is as described above, *e.g.*, for claim 75, see claims 46, 43, 45 and 51; for claim 76, see claims 51, 43, 45 and 62-64; for claim 77, see claim 51; and for claim 78, which refers to LTBP-3 only, see claims 51 and 71.

It will therefore be understood that no new matter is included within the present claims.

VI. Formalities

Applicants' representative, Shelley Fussey, has changed law firms since the parent application was filed. A new Power of Attorney was submitted in the parent application and an additional copy is enclosed herewith. All communications should be directed to the address listed therein and at the end of this document.

Formal drawings are included herewith.

No fees should be due in addition to the enclosed filing fees. However, should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason, the Assistant

Commissioner is authorized to deduct said fees from Williams, Morgan & Amerson, P.C.

Deposit Account No. 50-0786/4100.000582.

VII. Conclusion

The present claims are believed to be condition for allowance, and an early indication to this effect is respectfully requested. Should Examiner Fitzgerald have any questions or comments, a telephone call to the undersigned Applicants' representative is earnestly solicited.

Respectfully submitted,



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Date: June 12, 2000

EXHIBIT A
Pending Claims In Continuation

40. (Amended) A method for [identifying] binding a transforming growth factor β (TGF- β) protein in a sample, comprising contacting said sample with [an] a purified mammalian LTBP-2 or LTBP-3 protein or polypeptide under conditions effective to allow binding [and detecting the protein so bound] of said LTBP-2 or LTBP-3 protein or polypeptide to said TGF- β protein; wherein said LTBP-2 or LTBP-3 protein or polypeptide comprises at least fifteen contiguous amino acids present in SEQ ID NO:2 or SEQ ID NO:4, respectively.

43. The method of claim 40, wherein said sample is located within an animal and said LTBP-2 or LTBP-3 protein or polypeptide is administered to said animal in an amount effective to bind TGF- β in said animal.

44. A method of binding TGF- β , comprising contacting a composition comprising TGF- β with a composition comprising a purified mammalian LTBP-2 or LTBP-3 protein or polypeptide in an amount effective to bind TGF- β ; wherein said LTBP-2 or LTBP-3 protein or polypeptide comprises at least fifteen contiguous amino acids present in SEQ ID NO:2 or SEQ ID NO:4, respectively.

45. The method of claim 44, wherein said composition comprising TGF- β is located within an animal and said composition comprising said LTBP-2 or LTBP-3 protein or polypeptide is administered to said animal in an amount effective to bind TGF- β in said animal.

46. A method of using an LTBP-2 or LTBP-3 protein, polypeptide or peptide, comprising providing to an animal a biologically effective amount of a purified mammalian LTBP-2 or LTBP-3 protein, polypeptide or peptide that comprises at least fifteen contiguous amino acids present in SEQ ID NO:2 or SEQ ID NO:4, respectively.

47. The method of claim 46, wherein an amount of an LTBP-2 or LTBP-3 protein, polypeptide or peptide effective to generate anti-LTBP-2 or anti-LTBP-3 antibodies is provided to said animal.

48. The method of claim 47, wherein an LTBP-2 or LTBP-3 peptide of between 15 and about 50 amino acids in length is provided to said animal.

49. The method of claim 47, wherein an LTBP-2 or LTBP-3 peptide of between 15 and about 30 amino acids in length is provided to said animal.

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71. The method of claim 51, wherein said LTBP-2 or LTBP-3 protein or polypeptide exhibits at least 90% identity to the amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4, respectively.

72. The method of claim 51, wherein said LTBP-2 or LTBP-3 protein or polypeptide exhibits between 91% and about 99% identity to the amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4, respectively.

73. The method of claim 51, wherein an LTBP-2 protein comprising the amino acid sequence of SEQ ID NO:2 is provided to said animal.

74. The method of claim 51, wherein an LTBP-3 protein comprising the amino acid sequence of SEQ ID NO:4 is provided to said animal.

75. A method of using an LTBP-2 or LTBP-3 protein or polypeptide, comprising administering to an animal a purified mammalian LTBP-2 or LTBP-3 protein or polypeptide in an amount effective to bind TGF- β in said animal; wherein said LTBP-2 or LTBP-3 protein or polypeptide specifically binds TGF- β and comprises at least fifteen contiguous amino acids present in SEQ ID NO:2 or SEQ ID NO:4, respectively.

76. A method of binding TGF- β within a repair or bone progenitor tissue site of an animal, comprising contacting said tissue site with a purified mammalian LTBP-2 or LTBP-3 protein or polypeptide, or a nucleic acid that expresses said LTBP-2 or LTBP-3 protein or polypeptide, to provide an amount of said LTBP-2 or LTBP-3 protein or polypeptide effective to bind TGF- β in said animal; wherein said LTBP-2 or LTBP-3 protein or polypeptide comprises at least fifteen contiguous amino acids present in SEQ ID NO:2 or SEQ ID NO:4, respectively.

77. A method of binding TGF- β , comprising administering to an animal a composition comprising a purified mammalian LTBP-2 or LTBP-3 protein or polypeptide in an amount effective to bind TGF- β in said animal; wherein said LTBP-2 or LTBP-3 protein or polypeptide comprises at least fifteen contiguous amino acids present in SEQ ID NO:2 or SEQ ID NO:4, respectively.

78. A method of binding TGF- β , comprising administering to an animal a composition comprising a purified mammalian LTBP-3 protein or polypeptide in an amount effective to bind TGF- β in said animal; wherein said LTBP-3 protein or polypeptide binds TGF- β and comprises at least fifteen contiguous amino acids present in SEQ ID NO:4 or exhibits at least 90% identity to the amino acid sequence set forth in SEQ ID NO:4.

EXHIBIT B
Allowed Claims in Parent, Serial No. 08/479,722

127. An isolated nucleic acid molecule comprising a sequence encoding at least fifteen contiguous amino acids present in SEQ ID NO:4.

128. The isolated nucleic acid molecule of claim 127, comprising a sequence that encodes at least about thirty contiguous amino acids present in SEQ ID NO:4.

129. The isolated nucleic acid molecule of claim 127, comprising a sequence that encodes at least about fifty contiguous amino acids present in SEQ ID NO:4.

130. The isolated nucleic acid molecule of claim 127, comprising a sequence which, upon translation, affords a peptide of between 15 and about 50 amino acids in length.

131. The isolated nucleic acid molecule of claim 127, comprising a sequence which, upon translation, affords a peptide of between 15 and about 30 amino acids in length.

132. The isolated nucleic acid molecule of claim 127, comprising a sequence that encodes a polypeptide or peptide in which said at least fifteen contiguous amino acids present in SEQ ID NO:4 are present in domain 1, domain 2, domain 3, domain 4 or domain 5 of the LTBP-3 polypeptide sequence set forth in SEQ ID NO:4.

133. The isolated nucleic acid molecule of claim 132, comprising a sequence that encodes a polypeptide or peptide in which said at least fifteen contiguous amino acids present in SEQ ID NO:4 are present in domain 1 of the LTBP-3 polypeptide sequence set forth in SEQ ID NO:4.

134. The isolated nucleic acid molecule of claim 132, comprising a sequence that encodes a polypeptide or peptide in which said at least fifteen contiguous amino acids present in SEQ ID NO:4 are present in domain 2 of the LTBP-3 polypeptide sequence set forth in SEQ ID NO:4.

135. The isolated nucleic acid molecule of claim 132, comprising a sequence that encodes a polypeptide or peptide in which said at least fifteen contiguous amino acids present in SEQ ID NO:4 are present in domain 3 of the LTBP-3 polypeptide sequence set forth in SEQ ID NO:4.

136. The isolated nucleic acid molecule of claim 132, comprising a sequence that encodes a polypeptide or peptide in which said at least fifteen contiguous amino acids present in SEQ ID NO:4 are present in domain 4 of the LTBP-3 polypeptide sequence set forth in SEQ ID NO:4.

137. The isolated nucleic acid molecule of claim 132, comprising a sequence that encodes a polypeptide or peptide in which said at least fifteen contiguous amino acids present in SEQ ID NO:4 are present in domain 5 of the LTBP-3 polypeptide sequence set forth in SEQ ID NO:4.

138. The isolated nucleic acid molecule of claim 127, comprising a sequence that encodes a polypeptide comprising about 1,251 contiguous amino acids in SEQ ID NO:4.

140. (Amended) The isolated nucleic acid molecule of claim 138, comprising the contiguous nucleic acid sequence of SEQ ID NO:3.

141. The isolated nucleic acid molecule of claim 127, further comprising a recombinant promoter.

142. The isolated nucleic acid molecule of claim 141, wherein said sequence is positioned, in reverse orientation, under the control of a promoter that directs the expression of an antisense product.

143. The isolated nucleic acid molecule of claim 127, further defined as a recombinant vector.

144. An isolated nucleic acid molecule comprising a sequence that encodes a protein having the contiguous amino acid sequence of SEQ ID NO:4.

145. An isolated nucleic acid molecule encoding a polypeptide that exhibits at least 90% identity to the amino acid sequence set forth in SEQ ID NO:4, wherein said polypeptide specifically binds to TGF- β 1.

146. The isolated nucleic acid molecule of claim 145, wherein the encoded polypeptide exhibits between 91% and about 99% identity to the amino acid sequence set forth in SEQ ID NO:4.

147. An isolated nucleic acid molecule encoding a mammalian LTBP-3 polypeptide, wherein said nucleic acid molecule comprises the nucleotide sequence of:

- (a) the coding sequence of a cDNA molecule present in a mammalian library, wherein the cDNA molecule hybridizes with a probe having the sequence of the complement of SEQ ID NO:3 under conditions of high stringency; or
- (b) a nucleotide sequence degenerate with a sequence according to (a).

148. The isolated nucleic acid molecule of claim 147, wherein said nucleic acid molecule comprises the nucleotide sequence of the coding sequence of a cDNA molecule present in a mammalian library, wherein the cDNA molecule hybridizes with a probe having the sequence of the complement of SEQ ID NO:3 under conditions of high stringency.

149. The isolated nucleic acid molecule of claim 147, wherein said nucleic acid molecule comprises a nucleotide sequence degenerate with the coding sequence of a cDNA molecule present in a mammalian library, wherein the cDNA molecule hybridizes with a probe having the sequence of the complement of SEQ ID NO:3 under conditions of high stringency.

152. (Amended) The isolated nucleic acid molecule of claim 147, wherein the nucleic acid molecule comprises a nucleotide sequence of at least 50 contiguous nucleotides present in SEQ ID NO:3.

153. The isolated nucleic acid molecule of claim 152, wherein the nucleic acid molecule comprises a nucleotide sequence of at least 100 contiguous nucleotides present in SEQ ID NO:3.

154. The isolated nucleic acid molecule of claim 153, wherein the nucleic acid molecule comprises a nucleotide sequence of at least 200 contiguous nucleotides present in SEQ ID NO:3.

155. The isolated nucleic acid molecule of claim 154, wherein the nucleic acid molecule comprises a nucleotide sequence of at least 500 contiguous nucleotides present in SEQ ID NO:3.

156. The isolated nucleic acid molecule of claim 155, wherein the nucleic acid molecule comprises a nucleotide sequence of at least 1000 contiguous nucleotides present in SEQ ID NO:3.

157. The isolated nucleic acid molecule of claim 156, wherein the nucleic acid molecule comprises a nucleotide sequence of at least 3000 contiguous nucleotides present in SEQ ID NO:3.

159. The isolated nucleic acid molecule of claim 147, wherein the nucleic acid molecule is up to about 10,000 basepairs in length.

160. The isolated nucleic acid molecule of claim 159, wherein the nucleic acid molecule is up to about 5,000 basepairs in length.

161. The isolated nucleic acid molecule of claim 147, further defined as a DNA molecule.

162. The isolated nucleic acid molecule of claim 147, further defined as an RNA molecule.

163. An isolated nucleic acid molecule encoding a mammalian LTBP-3 polypeptide, wherein said nucleic acid molecule comprises the nucleotide sequence of

- (a) the coding sequence of a cDNA molecule present in a mammalian library, wherein the cDNA molecule hybridizes, under conditions of high stringency, with a probe having the sequence of the complement of the isolated LTBP-3 sequence region within the biological material deposited as ATCC 209496; or
- (b) a nucleotide sequence degenerate with a sequence according to (a).

164. (Amended) The isolated nucleic acid molecule of claim 163, wherein said nucleic acid molecule [encodes a mammalian LTBP-3 polypeptide that includes at least fifteen contiguous amino acids encoded by] comprises a sequence of at least 50 contiguous nucleotides present in the sequence of the isolated LTBP-3 sequence region within the biological material deposited as ATCC 209496.

165. The isolated nucleic acid molecule of claim 164, wherein said nucleic acid molecule encodes a mammalian LTBP-3 polypeptide that comprises about 1,251 contiguous amino acids encoded by the isolated LTBP-3 sequence region within the biological material deposited as ATCC 209496.

166. The isolated nucleic acid molecule of claim 165, wherein said nucleic acid molecule has the nucleotide sequence of the isolated LTBP-3 sequence region within the biological material deposited as ATCC 209496.

167. (Amended) An isolated nucleic acid molecule encoding a mammalian LTBP-3 polypeptide, wherein said nucleic acid molecule comprises the nucleotide sequence of

the coding sequence of a cDNA molecule present in a mammalian library, wherein the cDNA molecule hybridizes, under conditions of high stringency, with a probe having the sequence of the complement of the isolated LTBP-3 sequence region within the vector pLTBP-3fl, deposited as ATCC 209496.

168. The isolated nucleic acid molecule of claim 167, wherein said nucleic acid molecule encodes a mammalian LTBP-3 polypeptide that has the contiguous amino acid sequence encoded by the isolated LTBP-3 sequence region within the vector pLTBP-3fl.

169. A recombinant host cell comprising an isolated nucleic acid molecule in accordance with claim 127, claim 144, claim 145, claim 147, claim 163 or claim 167.

170. The recombinant host cell of claim 169, further defined as a prokaryotic host cell.

171. The recombinant host cell of claim 169, further defined as a eukaryotic host cell.

172. The recombinant host cell of claim 169, wherein the isolated nucleic acid molecule is introduced into the cell by means of a recombinant vector and the host cell expresses the isolated nucleic acid molecule to produce the encoded protein or peptide.

173. (Amended) A method of using an isolated nucleic acid molecule that encodes a protein or peptide, the method comprising expressing, in a recombinant host cell, a recombinant vector that comprises an isolated nucleic acid molecule in accordance with claim 127, claim 144, claim 145, claim 147, claim 163 or claim 167, and collecting the expressed protein or peptide.

EXHIBIT C
Claims Restricted from Parent, Serial No. 08/479,722

Group III:

22. A recombinant LTBP-2 or LTBP-3 protein or peptide prepared by expressing an LTBP-2- or LTBP-3-encoding DNA segment in a recombinant host cell and purifying the expressed LTBP-2- or LTBP-3 protein or peptide away from total recombinant host cell components.

42. A composition comprising a purified murine LTBP-2 or LTBP-3 polypeptide.

Part of Group IV

23. A method for detecting an LTBP-2 or LTBP-3 nucleic acid segment in a sample, comprising the steps of:

- (a) obtaining sample nucleic acids from a sample suspected of containing an LTBP-2 or LTBP-3 nucleic acid segment;
- (b) contacting said sample nucleic acids with an isolated LTBP-2 or LTBP-3 nucleic acid segment under conditions effective to allow hybridization of substantially complementary nucleic acids; and
- (c) detecting the hybridized complementary nucleic acids thus formed.

Group V:

37. A purified antibody that binds to an LTBP-3 protein or peptide.

38. The antibody of claim 37, wherein the antibody is linked to a detectable label.

39. An immunodetection kit comprising, in suitable container means, an LTBP-3 protein or peptide, or a first antibody that binds to an LTBP-3 protein or peptide, and an immunodetection reagent.

Group VI:

40. A method for identifying a transforming growth factor β protein in a sample, comprising contacting said sample with an LTBP-2 or LTBP-3 protein under conditions effective to allow binding and detecting the protein so bound.

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EXHIBIT D
Claims to Issue in Grandparent, Patent No. 5,942,496

51. (Amended) A method for transferring nucleic acid segments into bone progenitor cells located within a bone progenitor tissue site of an animal, comprising contacting said tissue site with a composition comprising two or more nucleic acid segments and a structural bone-compatible matrix, so as to transfer said two or more nucleic acid segments into said cells, wherein said cells express transcriptional or translational products encoded by said nucleic acid segments.

52. (Amended) The method of claim 51, comprising contacting bone progenitor cells with a composition comprising two nucleic acid segments and a structural bone-compatible matrix.

53. (Amended) The method of claim 51, comprising contacting bone progenitor cells with a composition comprising three nucleic acid segments and a structural bone-compatible matrix.

55. (Amended) The method of claim 51, wherein the contacting process comprises bringing said two or more nucleic acid segments into contact with said structural bone-compatible matrix to form a matrix-nucleic acid segments composition and bringing said matrix-nucleic acid segments composition into contact with said tissue site.

56. (Amended) The method of claim 55, wherein said nucleic acid segments are absorbed in said structural bone-compatible matrix.

57. (Amended) The method of claim 55, wherein said nucleic acid segments are adsorbed to said structural bone-compatible matrix.

58. (Amended) The method of claim 55, wherein said nucleic acid segments are impregnated within said structural bone-compatible matrix.

59. The method of claim 51, wherein said bone progenitor cells are stem cells, macrophages, fibroblasts, vascular cells, osteoblasts, chondroblasts or osteoclasts.

60. The method of claim 59, wherein said bone progenitor cells are fibroblasts.

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61. The method of claim 51, wherein at least one of said two or more nucleic acid segments is a DNA molecule.

62. (Amended) The method of claim 51, wherein at least one of said two or more nucleic acid segments is an antisense nucleic acid molecule.

63. The method of claim 51, wherein at least one of said two or more nucleic acid segments is a linear nucleic acid molecule, a plasmid or a recombinant insert within the genome of a recombinant virus.

64. (Amended) The method of claim 51, wherein at least one of said two or more nucleic acid segments encodes a polypeptide or protein that stimulates bone progenitor cells when expressed by said cells.

65. (Amended) The method of claim 51, wherein said structural bone-compatible matrix is a collagenous, metal, hydroxylapatite, bioglass, aluminate, bioceramic, acrylic ester polymer, lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

66. (Amended) The method of claim 65, wherein said structural bone-compatible matrix is a titanium matrix.

67. (Amended) The method of claim 66, wherein said structural bone-compatible matrix is a titanium matrix coated with hydroxylapatite.

68. (Amended) The method of claim 65, wherein said structural bone-compatible matrix is a collagen preparation.

69. (Amended) The method of claim 68, wherein said structural bone-compatible matrix is a type II collagen preparation.

70. (Amended) The method of claim 69, wherein said structural bone-compatible matrix is a recombinant type II collagen preparation.

71. (Amended) The method of claim 69, wherein said structural bone-compatible matrix is a type II collagen preparation further supplemented with minerals.

92. (Amended) The method of claim 73, wherein said structural bone-compatible matrix is a collagenous, metal, hydroxylapatite, bioglass, aluminate, bioceramic, acrylic ester polymer, lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.
93. (Amended) The method of claim 92, wherein said structural bone-compatible matrix is a titanium matrix.
94. (Amended) The method of claim 93, wherein said structural bone-compatible matrix is a titanium matrix coated with hydroxylapatite.
95. (Amended) The method of claim 92, wherein said structural bone-compatible matrix is a collagen preparation.
96. (Amended) The method of claim 95, wherein said structural bone-compatible matrix is a type II collagen preparation.
97. (Amended) The method of claim 96, wherein said structural bone-compatible matrix is a recombinant type II collagen preparation.
98. (Amended) The method of claim 96, wherein said structural bone-compatible matrix is a mineralized type II collagen preparation.
99. (Amended) The method of claim 92, wherein said structural bone-compatible matrix is a lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.
100. (Amended) The method of claim 73, wherein said composition is applied to a bone fracture site in said animal.
101. (Amended) The method of claim 73, wherein said composition is implanted within a bone cavity site in said animal.
102. The method of claim 101, wherein said bone cavity site is the result of dental or periodontal surgery or the removal of an osteosarcoma.

103. (Amended) A method of delivering two or more nucleic acid segments to a fibroblast cell located within a repair tissue site of an animal, comprising contacting said tissue site with a composition comprising two or more nucleic acid segments and a structural bone-compatible matrix to effect uptake of the nucleic acid segments into the fibroblast cell and to promote expression of transcriptional or translational products by said fibroblast cell.

104. (Amended) A method of delivering two or more selected nucleic acid segments to a fibroblast cell located within a repair tissue site of an animal, comprising the steps of:

- (a) preparing a matrix-nucleic acid composition comprising two or more nucleic acid segments and a structural bone-compatible matrix; and
- (b) contacting said repair tissue site with the structural matrix-nucleic acid composition to effect uptake of the nucleic acid segments by the fibroblast cell, wherein said fibroblast cell expresses transcriptional or translational products encoded by said nucleic acid segments.

105. (Amended) The method of claim 104, wherein step (a) comprises preparing a matrix-nucleic acid composition comprising two nucleic acid segments and a structural bone-compatible matrix.

106. (Amended) The method of claim 104, wherein step (a) comprises preparing a matrix-nucleic acid composition comprising three nucleic acid segments and a structural bone-compatible matrix.

108. (Amended) The method of claim 104, wherein said nucleic acid segments are absorbed in or adsorbed to said structural bone-compatible matrix.

109. (Amended) The method of claim 104, wherein said nucleic acid segments are impregnated within said structural bone-compatible matrix.

110. The method of claim 104, wherein at least one of said two or more nucleic acid segments is a DNA molecule.

111. The method of claim 104, wherein at least one of said two or more nucleic acid segments is an RNA molecule.

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123. The method of claim 122, wherein said matrix-nucleic acid composition comprises a PTH1-34 gene and a BMP-4 gene.

124. (Amended) The method of claim 104, wherein said structural bone-compatible matrix is a collagenous, metal, hydroxylapatite, hydroxylapatite-coated metal, bioglass, aluminate, bioceramic, acrylic ester polymer, lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

125. (Amended) The method of claim 124, wherein said structural bone-compatible matrix is a titanium matrix.

126. (Amended) The method of claim 125, wherein said structural bone-compatible matrix is a titanium matrix coated with hydroxylapatite.

127. (Amended) The method of claim 124, wherein said structural bone-compatible matrix is a collagen preparation.

128. (Amended) The method of claim 127, wherein said structural bone-compatible matrix is a type II collagen preparation.

129. (Amended) The method of claim 128, wherein said structural bone-compatible matrix is a recombinant type II collagen preparation.

130. (Amended) The method of claim 128, wherein said structural bone-compatible matrix is a type II collagen preparation further supplemented with minerals.

131. (Amended) The method of claim 124, wherein said structural bone-compatible matrix is a lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

132. (Amended) A composition comprising two or more nucleic acid segments in association with a structural bone-compatible matrix.

133. (Amended) The composition of claim 132, wherein said composition comprises two nucleic acid segments in association with said structural bone-compatible matrix.

134. (Amended) The composition of claim 132, wherein said composition comprises three nucleic acid segments in association with said structural bone-compatible matrix.

135. (Amended) The composition of claim 132, wherein said nucleic acid segments are absorbed in or adsorbed to said structural bone-compatible matrix.

136. (Amended) The composition of claim 132, wherein said nucleic acid segments are impregnated within said structural bone-compatible matrix.

137. The composition of claim 132, wherein at least one of said two or more nucleic acid segments is a DNA molecule.

138. The composition of claim 132, wherein at least one of said two or more nucleic acid segments is an RNA molecule.

139. The composition of claim 132, wherein at least one of said two or more nucleic acid segments is an antisense nucleic acid molecule.

140. The composition of claim 132, wherein at least one of said two or more nucleic acid segments is a linear nucleic acid molecule, a plasmid or a recombinant insert within the genome of a recombinant virus.

141. (Amended) The composition of claim 132, wherein at least one of said two or more nucleic acid segments encodes a polypeptide or protein that stimulates bone progenitor cells when expressed by said cells.

142. (Amended) The composition of claim 132, wherein said structural bone-compatible matrix is a collagenous, titanium, hydroxylapatite, hydroxylapatite-coated titanium, bioglass, aluminate, bioceramic, acrylic ester polymer, lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

143. (Amended) The composition of claim 142, wherein said structural bone-compatible matrix is a collagen preparation.

144. (Amended) The composition of claim 143, wherein said structural bone-compatible matrix is a type II collagen preparation.

145. (Amended) The composition of claim 144, wherein said structural bone-compatible matrix is a recombinant type II collagen preparation.

146. (Amended) The composition of claim 144, wherein said structural bone-compatible matrix is a type II collagen preparation further supplemented with minerals.

147. (Amended) The composition of claim 146, wherein said structural bone-compatible matrix is a type II collagen preparation further supplemented with calcium.

148. (Amended) The composition of claim 142, wherein said structural bone-compatible matrix is a lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

149. (Amended) A composition comprising two or more osteotropic genes in association with a structural bone-compatible matrix, said composition being capable of stimulating bone growth when administered to a bone progenitor tissue site of an animal.

150. (Amended) The composition of claim 149, wherein said composition comprises two osteotropic genes in association with said structural bone-compatible matrix.

151. (Amended) The composition of claim 149, wherein said composition comprises three osteotropic genes in association with said structural bone-compatible matrix.

152. (Amended) The composition of claim 149, wherein said osteotropic genes are absorbed in or adsorbed to said structural bone-compatible matrix.

153. (Amended) The composition of claim 149, wherein said osteotropic genes are impregnated within said structural bone-compatible matrix.

154. The composition of claim 149, wherein at least one of said two or more osteotropic genes is in the form of plasmid DNA, a DNA insert within the genome of a recombinant adenovirus, a DNA insert within the genome of a recombinant adeno-associated virus (AAV) or a DNA insert within the genome of a recombinant retrovirus.

155. (Amended) The composition of claim 149, wherein at least one of said two or more osteotropic genes is a PTH, BMP-2A, BMP-2B, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8, TGF- α , TGF- β 1, TGF- β 2, FGF, GMCSF, EGF, PDGF, IGF, LTBP or a LIF gene.

156. (Amended) The composition of claim 155, wherein at least one of said osteotropic genes is a TGF- α , TGF- β 1, TGF- β 2, PTH, LTBP, BMP-2 or BMP-4 gene.

157. The composition of claim 155, wherein at least one of said osteotropic genes is a PTH gene.

158. The composition of claim 157, wherein at least one of said osteotropic genes is a PTH1-34 gene.

159. The composition of claim 155, wherein at least one of said osteotropic genes is a BMP-2 or BMP-4 gene.

160. The composition of claim 155, wherein said composition comprises a PTH gene and a BMP gene.

161. The composition of claim 160, wherein said composition comprises a PTH1-34 gene and a BMP-4 gene.

162. (Amended) The composition of claim 149, wherein said structural bone-compatible matrix is a collagenous, metal, hydroxyapatite, bioglass, aluminate, bioceramic, acrylic ester polymer, lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

163. (Amended) The composition of claim 162, wherein said structural bone-compatible matrix is a titanium matrix.

164. (Amended) The composition of claim 163, wherein said structural bone-compatible matrix is a titanium matrix coated with hydroxylapatite.

165. (Amended) The composition of claim 162, wherein said structural bone-compatible matrix is a collagen preparation.

166. (Amended) The composition of claim 165, wherein said structural bone-compatible matrix is a type II collagen preparation.

167. (Amended) The composition of claim 166, wherein said structural bone-compatible matrix is a recombinant type II collagen preparation.

168. (Amended) The composition of claim 166, wherein said structural bone-compatible matrix is a type II collagen preparation further supplemented with minerals.

169. (Amended) The composition of claim 168, wherein said structural bone-compatible matrix is a type II collagen preparation further supplemented with calcium.

170. (Amended) The composition of claim 162, wherein said structural bone-compatible matrix is a lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

171. (Amended) An osteotropic device, comprising two or more osteotropic genes capable of expression by bone progenitor cells, the genes associated with an amount of a structural bone-compatible matrix effective to absorb or adsorb said genes, wherein said device is capable of stimulating bone formation when implanted within a bone progenitor tissue site of an animal.

172. The device of claim 171, wherein said device comprises three or more osteotropic genes.

173. The device of claim 171, wherein said device is a titanium or a hydroxylapatite-coated titanium device.

174. The device of claim 171, wherein said device is shaped to join a bone fracture site in said animal.

175. The device of claim 171, wherein said device is shaped to fill a bone cavity site in said animal.

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EXHIBIT E
Issued Claims in Great Grandparent, Patent No. 5,763,416

1. A method for transferring a nucleic acid segment into bone progenitor cells located within a bone progenitor tissue site of an animal, comprising contacting said tissue site with a composition comprising a nucleic acid segment and a structural bone-compatible matrix so as to transfer said nucleic acid segment into said cells, wherein said nucleic acid segment expresses a transcriptional or translational product in said cells.
2. The method of claim 1, wherein the contacting process comprises bringing said nucleic acid segment into contact with said structural bone-compatible matrix to form a matrix-nucleic acid segment composition and bringing said matrix-nucleic acid segment composition into contact with said tissue site.
3. The method of claim 2, wherein said nucleic acid segment is absorbed in said structural bone-compatible matrix.
4. The method of claim 2, wherein said nucleic acid segment is adsorbed to said structural bone-compatible matrix.
5. The method of claim 2, wherein said nucleic acid segment is impregnated within said structural bone-compatible matrix..
6. The method of claim 1, wherein said nucleic acid segment is a DNA molecule.
7. The method of claim 1, wherein said nucleic acid segment is an RNA molecule.
8. The method of claim 1, wherein said nucleic acid segment is an antisense nucleic acid molecule.
9. The method of claim 1, wherein said nucleic acid segment is a linear nucleic acid molecule, a plasmid or a recombinant insert within the genome of a recombinant virus.
10. The method of claim 1, wherein said nucleic acid segment encodes a polypeptide or protein that stimulates bone progenitor cells when expressed in said cells.

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11. The method of claim 1, wherein said structural bone-compatible matrix is a collagenous, metal, hydroxylapatite, bioglass, aluminate, bioceramic, acrylic ester polymer, lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

12. The method of claim 11, wherein said structural bone-compatible matrix is a titanium matrix.

13. The method of claim 12, wherein said structural bone-compatible matrix is a titanium matrix coated with hydroxylapatite.

14. The method of claim 11, wherein said bone-compatible matrix is a collagen preparation.

15. The method of claim 11, wherein said bone-compatible matrix is a lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

16. The method of claim 1, wherein said bone progenitor cells are stem cells, macrophages, fibroblasts, vascular cells, osteoblasts, chondroblasts or osteoclasts.

17. The method of claim 16, wherein said bone progenitor cells are fibroblasts.

18. A method of stimulating bone progenitor cells located within a bone progenitor tissue site of an animal, comprising contacting said tissue site with a composition comprising an osteotropic gene and a structural bone-compatible matrix so as to promote expression of said gene in said cells.

19. The method of claim 18, wherein expression of said gene in said cells stimulates said cells to promote bone tissue growth.

20. The method of claim 18, wherein the contacting process comprises bringing said osteotropic gene into contact with said structural bone-compatible matrix to form a matrix-gene composition and bringing said matrix-gene composition into contact with said tissue site.

21. The method of claim 20, wherein said nucleic acid segment is absorbed in said structural bone-compatible matrix.

32. The method of claim 18, wherein said structural bone-compatible matrix is a collagenous, metal, hydroxylapatite, bioglass, aluminate, bioceramic, acrylic ester polymer, lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

33. The method of claim 32, wherein said structural bone-compatible matrix is a titanium matrix.

34. The method of claim 33, wherein said structural bone-compatible matrix is a titanium matrix coated with hydroxylapatite.

35. The method of claim 32, wherein said structural bone-compatible matrix is a collagen preparation.

36. The method of claim 32, wherein said structural bone-compatible matrix is a lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

37. The method of claim 19, wherein said matrix-gene composition is applied to a bone fracture site in said animal.

38. The method of claim 19, wherein said matrix-gene composition is implanted within a bone cavity site in said animal.

39. The method of claim 38, wherein said bone cavity site is the result of dental or periodontal surgery or the removal of an osteosarcoma.

40. The method of claim 18, wherein said bone progenitor cells are stem cells, macrophages, fibroblasts, vascular cells, osteoblasts, chondroblasts or osteoclasts.

41. The method of claim 40, wherein said bone progenitor cells are fibroblasts.

42. A composition comprising a nucleic acid segment in association with a structural bone-compatible matrix.

43. The composition of claim 42, wherein said nucleic acid segment is absorbed in said structural bone-compatible matrix.

44. The composition of claim 42, wherein said nucleic acid segment is adsorbed to said structural bone-compatible matrix.

45. The composition of claim 42, wherein said nucleic acid segment is impregnated within said structural bone-compatible matrix.

46. The composition of claim 42, wherein said nucleic acid segment is a DNA molecule.

47. The composition of claim 42, wherein said nucleic acid segment is an RNA molecule.

48. The composition of claim 42, wherein said nucleic acid segment is an antisense nucleic acid molecule.

49. The composition of claim 42, wherein said nucleic acid segment is a linear nucleic acid molecule, a plasmid or a recombinant insert within the genome of a recombinant virus.

50. The composition of claim 42, wherein said nucleic acid segment encodes a polypeptide or protein that stimulates bone progenitor cells when expressed in said cells.

51. The composition of claim 42, wherein said structural bone-compatible matrix is a collagenous, titanium, hydroxylapatite, hydroxylapatite-coated titanium, bioglass, aluminate, bioceramic, acrylic ester polymer, lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

52. The composition of claim 51, wherein said structural bone-compatible matrix is a collagen preparation.

53. The composition of claim 51, wherein said bone-compatible matrix is a lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

54. A composition comprising an osteotropic gene in association with a bone-compatible matrix, said composition being capable of stimulating bone growth when administered to a bone progenitor tissue site of an animal.

55. The composition of claim 54, wherein said osteotropic gene is absorbed in said structural bone-compatible matrix.

56. The composition of claim 54, wherein said osteotropic gene is adsorbed to said structural bone-compatible matrix.

57. The composition of claim 54, wherein said osteotropic gene is impregnated within said structural bone-compatible matrix.

58. The composition of claim 54, wherein said osteotropic gene is in the form of plasmid DNA, a DNA insert within the genome of a recombinant adenovirus, a DNA insert within the genome of a recombinant adeno-associated virus (AAV) or a DNA insert within the genome of a recombinant retrovirus.

59. The composition of claim 54, wherein said osteotropic gene is a PTH, BMP-2A, BMP-2B, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8, TGF- α , TGF- β 1, TGF- β 2, FGF, GMCSF, EGF, PDGF, IGF or a LIF gene.

60. The composition claim 59, wherein said osteotropic gene is a TGF- α , TGF- β 1, TGF- β 2, PTH, BMP-2 or BMP-4 gene.

61. The composition of claim 60, wherein said osteotropic gene is a PTH1-34 gene.

62. The composition of claim 54, wherein said structural bone-compatible matrix is a collagenous, metal, hydroxyapatite, bioglass, aluminate, bioceramic, acrylic ester polymer, lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

63. The composition of claim 62, wherein said structural bone-compatible matrix is a titanium matrix.

64. The composition of claim 63, wherein said structural bone-compatible matrix is a titanium matrix coated with hydroxylapatite.

65. The composition of claim 62, wherein said structural bone-compatible matrix is a collagen preparation.

66. The composition of claim 62, wherein said structural bone-compatible matrix is a lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

67. An osteotropic device, comprising an osteotropic gene capable of expression in bone progenitor cells, the gene associated with an amount of a structural bone-compatible matrix effective to absorb said gene, wherein said device is capable of stimulating bone formation when implanted within a bone progenitor tissue site of an animal.

68. The device of claim 67, wherein said device is a titanium or a hydroxylapatite-coated titanium device.

69. The device of claim 67, wherein said device is shaped to join a bone fracture site in said animal.

70. The device of claim 67, wherein said device is shaped to fill a bone cavity site in said animal.

71. The device of claim 67, wherein said device is an artificial joint.

72. A method of delivering a nucleic acid segment to a fibroblast cell located within a repair tissue site of an animal, comprising contacting said tissue site with a composition comprising a nucleic acid segment and a structural bone-compatible matrix to effect uptake of the nucleic acid segment into the fibroblast cell and to promote expression of a transcriptional or translational product in said cell.

73. A method of delivering at least one selected nucleic acid segment to a fibroblast cell located within a repair tissue site of an animal, comprising the steps of:

- (a) preparing a matrix-nucleic acid composition comprising at least one nucleic acid segment and a structural bone-compatible matrix; and
- (b) contacting said repair tissue site with the structural matrix-nucleic acid composition to effect uptake of the nucleic acid segment by the fibroblast cell, wherein said nucleic acid segment expresses a transcriptional or translational product in said cell.

74. The method of claim 73, wherein said nucleic acid segment is a linear nucleic acid molecule, a plasmid or a recombinant insert within the genome of a recombinant virus.

75. The method of claim 73, wherein said structural bone-compatible matrix is a collagenous, metal, hydroxylapatite, hydroxylapatite-coated metal, bioglass, aluminate, bioceramic, acrylic ester polymer, lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

76. The method of claim 75, wherein said structural bone-compatible matrix is a collagenous matrix.

77. The method of claim 75, wherein said structural bone-compatible matrix is a lactic acid polymer, glycolic acid polymer or lactic acid/glycolic acid polymer matrix.

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1. A method for transferring a nucleic acid segment into bone progenitor cells, comprising contacting bone progenitor cells with a composition comprising a nucleic acid segment so as to transfer said nucleic acid segment into said cells.
2. The method of claim 1, wherein said cells are located within a bone progenitor tissue site of an animal and said tissue site is contacted with said composition so as to promote nucleic acid transfer into bone progenitor cells *in situ*.
3. The method of claim 2, wherein the contacting process comprises bringing said nucleic acid segment into contact with a bone-compatible matrix to form a matrix-nucleic acid segment composition and bringing said matrix-nucleic acid segment composition into contact with said tissue site.
4. The method of claim 1, wherein said nucleic acid segment is a DNA molecule.
5. The method of claim 1, wherein said nucleic acid segment is an RNA molecule.
6. The method of claim 1, wherein said nucleic acid segment is an antisense nucleic acid molecule.
7. The method of claim 1, wherein said nucleic acid segment is a linear nucleic acid molecule, a plasmid or a recombinant insert within the genome of a recombinant virus.
8. The method of claim 1, wherein said nucleic acid segment encodes a polypeptide or protein that stimulates bone progenitor cells when expressed in said cells.
9. The method of claim 3, wherein said bone-compatible matrix is a collagenous, metal, hydroxylapatite, bioglass, aluminate, bioceramic, acrylic ester polymer or lactic acid polymer matrix.
10. The method of claim 9, wherein said bone-compatible matrix is a titanium matrix.

11. The method of claim 10, wherein said bone-compatible matrix is a titanium matrix coated with hydroxylapatite.

12. The method of claim 9, wherein said bone-compatible matrix is a collagen preparation.

13. A method of stimulating bone progenitor cells, comprising contacting bone progenitor cells with a composition comprising an osteotropic gene so as to promote expression of said gene in said cells.

14. The method of claim 13, wherein said cells are located within a bone progenitor tissue site of an animal and said tissue site is contacted with said composition so as to promote bone tissue growth.

15. The method of claim 14, wherein the contacting process comprises bringing said osteotropic gene into contact with a bone-compatible matrix to form a matrix-gene composition and bringing said matrix-gene composition into contact with said tissue site.

16. The method of claim 13, wherein said osteotropic gene is in the form of plasmid DNA, a DNA insert within the genome of a recombinant adenovirus, a DNA insert within the genome of a recombinant adeno-associated virus (AAV) or a DNA insert within the genome of a recombinant retrovirus.

17. The method of claim 13, wherein said osteotropic gene is a parathyroid hormone (PTH) gene, a bone morphogenetic protein (BMP) gene, a growth factor gene, a growth factor receptor gene, a cytokine gene or a chemotactic factor gene.

18. The method of claim 17, wherein said osteotropic gene is a transforming growth factor (TGF) gene, a fibroblast growth factor (FGF) gene, a granulocyte/macrophage colony stimulating factor (GM-CSF) gene, an epidermal growth factor (EGF) gene, a platelet derived growth factor (PDGF) gene, an insulin-like growth factor (IGF) gene, or a leukemia inhibitory factor (LIF) gene.

19. The method of claim 18, wherein said osteotropic gene is a TGF- α , TGF- β 1 or TGF- β 2 gene.

20. The method of claim 17, wherein said osteotropic gene is a PTH gene.

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21. The method of claim 17, wherein said osteotropic gene is a BMP-2A, BMP-2B, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7 or BMP-8 gene.

22. The method of claim 21, wherein said osteotropic gene is a BMP-2 or BMP-4 gene.

23. The method of claim 15, wherein said bone-compatible matrix is a collagenous, metal, hydroxylapatite, bioglass, aluminate, bioceramic, acrylic ester polymer or lactic acid polymer matrix.

24. The method of claim 23, wherein said bone-compatible matrix is a titanium matrix.

25. The method of claim 24, wherein said bone-compatible matrix is a titanium matrix coated with hydroxylapatite.

26. The method of claim 23, wherein said bone-compatible matrix is a collagen preparation.

27. The method of claim 15, wherein said matrix-gene composition is applied to a bone fracture site in said animal.

28. The method of claim 15, wherein said matrix-gene composition is implanted within a bone cavity site in said animal.

29. The method of claim 15, wherein said bone cavity site is the result of dental or periodontal surgery or the removal of an osteosarcoma.

30. A composition comprising a nucleic acid segment in association with a bone-compatible matrix.

31. The composition of claim 30, wherein said nucleic acid segment is a DNA molecule.

32. The composition of claim 30, wherein said nucleic acid segment is an RNA molecule.

By

and

Wushan Yin

NUMBER TB900062031US

DATE OF DEPOSIT June 7, 1995

I hereby certify that this paper or fee is being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to The Commissioner of Patents and Trademarks, Washington, D.C. 20231.

SHELLEY P.M. FUSSEY

BACKGROUND OF THE INVENTION

The present application is a continuation-in-part of PCT/US95/02251, filed February 21, 1995; which is a continuation-in-part of U.S. Serial Number 08/316,650, filed September 30, 1994; which is a continuation-in-part of U.S. Serial Number 08/199,780, filed February 18, 1994; the entire text and figures of which disclosures are specifically incorporated herein by reference without disclaimer. The United States government has certain rights in the present invention pursuant to Grant HL-41926 from the National Institutes of Health.

A. Field of the Invention

The present invention relates generally to the field of molecular biology. More particularly it relates to latent TGF β binding protein (LTBP) genes, compositions and methods of use.

B. Description of the Related Art

1. TGF- β

Five TGF- β family members, which share 66-82% sequence identity, have been identified (Kingsley, 1994). Whereas TGF- β 1 was cloned from a cDNA library derived from human placenta, TGF- β 2 was subsequently purified from several mammalian cells and tissues, and TGF- β 3, - β 4, and - β 5 were cloned by low stringency hybridization from mammalian, avian and amphibian cDNA libraries, respectively. Peptide growth factors/cytokines have also been identified that share sequence homology ($\leq 40\%$) with the TGF- β s (collectively, the TGF- β s plus these other cytokines make up the TGF- β superfamily). A unifying feature of the biology of these other cytokines (*i.e.*, the Mullerian inhibiting substance, bone morphogenetic proteins, growth and differentiation factors, activin/inhibin, *Drosophila* decapentaplegic complex, and amphibian Vg1 protein) is the ability to regulate developmental processes. In every case where information is available,

superfamily members are synthesized as larger precursors that are processed at endoproteolytic cleavage motifs, and they terminate with the sequence C-X-C-X. The three dimensional crystal structure of the TGF- β 2 homodimer was recently reported (McDonald and Hendrickson, 1993). This work has led to the interesting and novel suggestion that TGF- β is related to certain peptide growth factors (*e.g.*, NGF, PDGF, v-SIS) in a way that could not have been predicted from the deduced amino acid analysis.

2. Latent TGF- β Complexes

Many cell types produce TGF- β , and almost all cells bind TGF- β with affinities in the picomolar range — *e.g.*, the type I and type II TGF- β cell surface receptors (glycoproteins of 53 and 75 kDa, respectively) are present in essentially all cells (Miyazono *et al.*, 1994). Thus, TGF- β has powerful effects on most cell types, and cytokines such as TGF- β are thought to exert broad control the tissue remodeling that occurs during development, wound repair, and other situations (Sporn *et al.*, 1986; Moses *et al.*, 1990). (For a comprehensive review of TGF- β effects, see Roberts and Sporn, 1990). For example, TGF- β was initially identified as a factor that stimulated the anchorage independent growth of rodent fibroblasts (Assoian *et al.*, 1983; Frolik *et al.*, 1983; Roberts *et al.*, 1983). It is now known, however, that TGF- β acts as a potential growth inhibitor for most cells, *i.e.*, epithelial, endothelial, and hematopoietic progenitor cells; both stimulates and inhibits cellular differentiation; induces extracellular matrix production by stimulating the expression of matrix macromolecules, stimulating the expression of matrix protease inhibitors, and decreasing the expression of matrix degrading proteases; inhibits the functional activities of immune cells; induces the chemotaxis of fibroblasts, macrophages, and smooth muscle cells; induces angiogenesis *in vivo*; inhibits endothelial migration; induces the expression of cell surface receptors for other cytokines (*e.g.*, the EGF receptor); promotes the healing of incisional wounds; inhibits osteoblast proliferation *in vitro*; and induces new bone formation *in vivo*.

A molecular explanation for these complex (and, at times, conflicting) effects is not

yet available, but hypotheses do exist. Sporn *et al.* (1986) have suggested, for example, that the ability of TGF- β to stimulate or inhibit the proliferation of mesenchymal cells depends on the state of cellular differentiation and the entire set of growth factors operant in that cell population. As such, the "biological meaning" of TGF- β signal transduction depends on the context (*i.e.*, availability and presentation) of other growth factors present in the local environment: Fischer rat 3T3 cells transfected with a *myc* gene and incubated with TGF- β and PDGF proliferate in soft agar, whereas the same cells in the presence of TGF- β and EGF fail to grow (Roberts *et al.*, 1985).

Whatever the mechanism, the autocrine and paracrine activities of TGF- β clearly must be regulated with precision. One regulatory strategy involves the temporal and spatial control of TGF- β gene expression. A second strategy involves the production and storage of TGF- β as a latent complex that is activated only under certain physiological and pathological conditions — *e.g.*, tissue morphogenesis and remodeling, and wound healing. TGF- β 1 can be isolated from serum and from most tissues as a latent complex (Pircher *et al.*, 1986; Miyazono *et al.*, 1988; Wakefield *et al.*, 1988). In this regard, the latent complex has been purified from human platelets and characterized in detail (Miyazono *et al.*, 1988). Following a 6-step protocol, the purified complex yielded protein bands of Mr 25,000 and 210,000 on SDS-PAGE under nonreducing conditions. After reduction, the 25 kDa band was shown to consist of subunits of Mr 12,500. On the other hand, the 210 kDa band consisted of a Mr 40,000 subunit and Mr 125-160,000 subunit.

TGF- β is also secreted from several producer cell lines in culture as a latent complex of 235 kDa (Gentry *et al.*, 1987). TGF- β 1 is initially synthesized *in vitro* as a 390 amino acid precursor that consists of a signal peptide, an amino-terminal propeptide, and the mature growth factor. Two precursor chains associate to form a disulfide-bonded dimer with latent activity. Homodimers occur most commonly, but heterodimers may also form (Ogawa *et al.*, 1992). The full length dimer is cleaved at a endoproteolytic cleavage motif, but the propeptide dimer (*i.e.*, the latency associated peptide or LAP) and the mature growth factor

dimer typically remain non-covalently associated. The mature TGF- β dimer is now known to be the 25 kDa band identified after nonreducing SDS-PAGE of the purified latent complex from platelets. In addition, LAP is known to be a component of the 210 kDa band identified after nonreducing SDS-PAGE of the purified latent complex from platelets (*i.e.*, LAP has been shown to consist of two of the 40 kDa subunits).

Together LAP and the mature TGF- β dimer form the small latent complex. As demonstrated in platelets, small latent complexes may be associated with additional high molecular weight proteins, the best characterized of which is the latent TGF- β binding protein or LTBP (Kanzaki *et al.*, 1990). (LTBP has been shown to be the 125-160 kDa subunit of the purified latent complex from platelets). Latent TGF- β complexes that contain LTBP are also known as large latent complexes. In contrast to platelet LTBP, the LTBP produced by fibroblasts typically is a 190 kDa polypeptide. The smaller size of platelet LTBP may be due to proteolytic processing or alternative splicing (Kanzaki *et al.*, 1990; Tsuji *et al.*, 1990).

3. LAP and Latency

TGF- β latency results in part from the non-covalent association of the propeptide dimer and the mature TGF- β dimer (Pircher *et al.*, 1984; Gentry *et al.*, 1988; Wakefield *et al.*, 1989). A cDNA for the TGF- β 1 precursor was expressed in Chinese Hamster Ovary (CHO) cells, which do not express LTBP (Gentry *et al.*, 1988), and almost all TGF- β activity recovered from the medium of transfected cells was latent. Use of deletion constructs has demonstrated that synthesis of biologically active TGF- β 1 can proceed only from the first ATG codon, implicating LAP in the proper assembly of the small latent complex in these cells. Taken together, these studies indicate that LAP is sufficient to achieve the latent state. More recent studies have shown that carbohydrate structures within LAP make an important contribution to the latent state. For example, treatment of the latent form of TGF- β 1 with endoglycosidase F led to activation of TGF- β (Miyazono and Heldin,

1989). (The structure of the mature TGF- β dimer was not affected by enzyme treatment). In particular, sialic acid residues seemed to be important, as treatment of the purified latent complex with sialidase was also able to activate TGF- β from the latent state.

5 4. Modulation of Latency

Latent complexes must be dissociated to activate mature TGF- β , and dissociation is considered to be a critical step in governing TGF- β effects (Twardzik *et al.*, 1990; Sato *et al.*, 1993). Dissociation by chemical treatment of the latent complex purified from platelets has been investigated (Miyazono *et al.*, 1990). Incubation of the purified complex under conditions of varying pH revealed that TGF- β activity was unmasked at values below pH 3.5 and above pH 12.5. Incubation of latent TGF- β in 0.02% SDS or 8 M urea also effectively unmasked TGF- β activity, but incubation in 5 M NaCl did not. Wakefield *et al.* (Wakefield *et al.*, 1989) have reported that, after activation, TGF- β 1 and LAP reassociate in a time- and concentration-dependent manner under neutral, nondenaturing conditions. These results are consistent with the idea that the mature TGF- β dimer is non-covalently associated with LAP.

Latent TGF- β complexes are also dissociated by the action of certain enzymes. For example, latent TGF- β is activated by plasmin, which disrupts the structure of the large latent complex (Lyons *et al.*, 1988; Taipale *et al.*, 1995). Similar data exist for other enzymes, *e.g.*, cathepsin D, mast cell chymase, leukocyte elastase, and the glycosidases. Recently, osteoclast-derived cells were shown to be capable of activating latent TGF- β *in vitro* (Oreffo *et al.*, 1989). Osteoclast activation is of particular interest because of the hypothesis that TGF- β serves as a link between bone turnover and formation during bone remodeling (Centrella *et al.*, 1991). The mechanism of TGF- β activation by osteoclasts is not known at present, but it is reasonable to think that local alteration of pH due to action of proton pumps in the osteoclast plasma membrane or the release of osteoclast-derived proteases may be involved in the activation process. Related to these observations, activated macrophages (as might be found at a wound site or during tissue morphogenesis) secrete

sialidase and other proteases (Pilatte *et al.*, 1987), and they can lower the local pH to 4.0 (Silver *et al.*, 1988), both of which could contribute to TGF- β activation *in vivo*. As mentioned above, acidification weakens the non-covalent interaction between LAP and the mature TGF- β dimer (Wakefield *et al.*, 1989).

5

SUMMARY OF THE INVENTION

The present invention concerns in an overall and general sense novel DNA segments and recombinant vectors encoding LTBP-2 or LTBP-3, and the creation and use of recombinant host cells through the application of DNA technology, that express LTBP-2 or LTBP-3 gene products. As such, the invention concerns DNA segment comprising an isolated gene that encodes a protein or peptide that includes an amino acid sequence essentially as set forth by a contiguous sequence from SEQ ID NO:2 or SEQ ID NO:4. These DNA segments are represented by those that include a nucleic acid sequence essentially as set forth by a contiguous sequence from SEQ ID NO:1 or SEQ ID NO:3.

Compositions that include a purified protein that has an amino acid sequence essentially as set forth by the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 are also encompassed by the invention.

The TGF- β s represent a family of structurally related molecules with diverse effects on mammalian cell shape, growth, and differentiation (Roberts and Sporn, 1990). Initially synthesized as a precursor consisting of an amino-terminal propeptide followed by mature TGF- β , two chains of nascent pro-TGF- β associate in most tissues to form a Mr ~106,000 inactive disulfide-bonded dimer. Homodimers are most common, but heterodimers have also been described (Cheifetz *et al.*, 1987; Ogawa *et al.*, 1992). During biosynthesis the mature TGF- β dimer is cleaved from the propeptide dimer. TGF- β latency results in part from the non-covalent association of propeptide and mature TGF- β dimers (Pircher *et al.*, 1984, 1986; Wakefield *et al.*, 1987; Millan *et al.*, 1992; Miyazono and Heldin, 1989). Consequently, the

propeptide dimer is often referred to as the latency associated protein (LAP), and LAP plus the disulfide-bonded TGF- β dimer are also known as the small latent complex. In the extracellular space small latent complexes must be dissociated to activate mature TGF- β . The mechanism of activation of the latent complex is thought to be one of the most important steps governing TGF- β effects (Lyons *et al.*, 1988; Antonelli-Orlidge *et al.*, 1989; Twardzik *et al.*, 1990; Sato *et al.*, 1993).

In certain lines of cultured cells small latent growth factor complexes may contain additional high molecular weight proteins. The best characterized of these high molecular weight proteins is the latent TGF- β binding protein, or LTBP (Miyazono *et al.*, 1988; Kanzaki *et al.*, 1990; Tsuji *et al.*, 1990; Olofsson *et al.*, 1992; Taketazu *et al.*, 1994). LTBP produced by different cell types is heterogeneous in size, perhaps because of alternative splicing or because of tissue-specific proteolytic processing (Miyazono *et al.*, 1988; Wakefield *et al.*, 1988; Kanzaki *et al.*, 1990; Tsuji *et al.*, 1990). Latent TGF- β complexes that contain LTBP are known as large latent complexes. LTBP has no known covalent linkage to mature TGF- β , but rather it is linked by a disulfide bond to LAP.

Regarding the novel protein LTBP-2 or LTBP-3, the present invention concerns DNA segments, that can be isolated from virtually any mammalian source, that are free from total genomic DNA and that encode proteins having LTBP-2-like or LTBP-3-like activity. DNA segments encoding LTBP-2-like or LTBP-3-like species may prove to encode proteins, polypeptides, subunits, functional domains, and the like.

As used herein, the term "DNA segment" refers to a DNA molecule that has been isolated free of total genomic DNA of a particular species. Therefore, a DNA segment encoding LTBP-2 or LTBP-3 refers to a DNA segment that contains LTBP-2 or LTBP-3 coding sequences yet is isolated away from, or purified free from, total genomic DNA of the species from which the DNA segment is obtained. Included within the term "DNA segment", are DNA segments and smaller fragments of such segments, and also recombinant

vectors, including, for example, plasmids, cosmids, phagemids, phage, viruses, and the like.

Similarly, a DNA segment comprising an isolated or purified LTBP-2 or LTBP-3 gene refers to a DNA segment including LTBP-2 or LTBP-3 coding sequences and, in certain aspects, regulatory sequences, isolated substantially away from other naturally occurring genes or protein encoding sequences. In this respect, the term "gene" is used for simplicity to refer to a functional protein, polypeptide or peptide encoding unit. As will be understood by those in the art, this functional term includes both genomic sequences, cDNA sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides or peptides.

"Isolated substantially away from other coding sequences" means that the gene of interest, in this case, a gene encoding LTBP-2 or LTBP-3, forms the significant part of the coding region of the DNA segment, and that the DNA segment does not contain large portions of naturally-occurring coding DNA, such as large chromosomal fragments or other functional genes or cDNA coding regions. Of course, this refers to the DNA segment as originally isolated, and does not exclude genes or coding regions later added to the segment by the hand of man.

In particular embodiments, the invention concerns isolated DNA segments and recombinant vectors incorporating DNA sequences that encode an LTBP-2 or LTBP-3 species that includes within its amino acid sequence an amino acid sequence essentially as set forth in SEQ ID NO:2 or SEQ ID NO:4. In other particular embodiments, the invention concerns isolated DNA segments and recombinant vectors incorporating DNA sequences that include within their sequence a nucleotide sequence essentially as set forth in SEQ ID NO:1 or SEQ ID NO:3.

The term "a sequence essentially as set forth in SEQ ID NO:2 or SEQ ID NO:4" means that the sequence substantially corresponds to a portion of SEQ ID NO:2 or SEQ ID NO:4 and has relatively few amino acids that are not identical to, or a biologically functional

equivalent of, the amino acids of SEQ ID NO:2 or SEQ ID NO:4. The term "biologically functional equivalent" is well understood in the art and is further defined in detail herein (for example, see section 7, preferred embodiments). Accordingly, sequences that have between about 70% and about 80%; or more preferably, between about 81% and about 90%; or even more preferably, between about 91% and about 99%; of amino acids that are identical or functionally equivalent to the amino acids of SEQ ID NO:2 or SEQ ID NO:4 will be sequences that are "essentially as set forth in SEQ ID NO:2 or SEQ ID NO:4".

In certain other embodiments, the invention concerns isolated DNA segments and recombinant vectors that include within their sequence a nucleic acid sequence essentially as set forth in SEQ ID NO:1 or SEQ ID NO:3. The term "essentially as set forth in SEQ ID NO:1 or SEQ ID NO:3" is used in the same sense as described above and means that the nucleic acid sequence substantially corresponds to a portion of SEQ ID NO:1 or SEQ ID NO:3 and has relatively few codons that are not identical, or functionally equivalent, to the codons of SEQ ID NO:1 or SEQ ID NO:3. Again, DNA segments that encode proteins exhibiting LTBP-2-like or LTBP-3-like activity will be most preferred.

It will also be understood that amino acid and nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids or 5' or 3' sequences, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological protein activity where protein expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences flanking either of the 5' or 3' portions of the coding region or may include various internal sequences, *i.e.*, introns, which are known to occur within genes.

Naturally, the present invention also encompasses DNA segments that are complementary, or essentially complementary, to the sequence set forth in SEQ ID NO:1 or SEQ ID NO:3. Nucleic acid sequences that are "complementary" are those that are capable of base-pairing according to the standard Watson-Crick complementarity rules. As used

herein, the term "complementary sequences" means nucleic acid sequences that are substantially complementary, as may be assessed by the same nucleotide comparison set forth above, or as defined as being capable of hybridizing to the nucleic acid segment of SEQ ID NO:1 or SEQ ID NO:3, under relatively stringent conditions such as those described herein.

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The nucleic acid segments of the present invention, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, nucleic acid fragments may be prepared that include a short contiguous stretch identical to or complementary to SEQ ID NO:1 or SEQ ID NO:3, such as about 14 nucleotides, and that are up to about 10,000 or about 5,000 base pairs in length, with segments of about 3,000 being preferred in certain cases. DNA segments with total lengths of about 1,000, about 500, about 200, about 100 and about 50 base pairs in length (including all intermediate lengths) are also contemplated to be useful.

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It will be readily understood that "intermediate lengths", in these contexts, means any length between the quoted ranges, such as 14, 15, 16, 17, 18, 19, 20, *etc.*; 21, 22, 23, *etc.*; 30, 31, 32, *etc.*; 50, 51, 52, 53, *etc.*; 100, 101, 102, 103, *etc.*; 150, 151, 152, 153, *etc.*; including all integers through the 200-500; 500-1,000; 1,000-2,000; 2,000-3,000; 3,000-5,000; 5,000-10,000 ranges, up to and including sequences of about 12,001, 12,002, 13,001, 13,002 and the like.

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It will also be understood that this invention is not limited to the particular nucleic acid and amino acid sequences of SEQ ID NO:1 or SEQ ID NO:3; and SEQ ID NO:2 or SEQ ID NO:4, respectfully. Recombinant vectors and isolated DNA segments may therefore variously include the LTBP-2 or LTBP-3 coding regions themselves, coding regions bearing selected alterations or modifications in the basic coding region, or they may encode larger

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polypeptides that nevertheless include LTBP-2 or LTBP-3-coding regions or may encode biologically functional equivalent proteins or peptides that have variant amino acids sequences.

5 The DNA segments of the present invention encompass biologically functional equivalent LTBP-2 or LTBP-3 proteins and peptides. Such sequences may arise as a consequence of codon redundancy and functional equivalency that are known to occur naturally within nucleic acid sequences and the proteins thus encoded. Alternatively, functionally equivalent proteins or peptides may be created via the application of recombinant
10 DNA technology, in which changes in the protein structure may be engineered, based on considerations of the properties of the amino acids being exchanged. Changes designed by man may be introduced through the application of site-directed mutagenesis techniques, *e.g.*, to introduce improvements to the antigenicity of the protein or to test mutants in order to examine activity at the molecular level.

 If desired, one may also prepare fusion proteins and peptides, *e.g.*, where the LTBP-2 or LTBP-3 coding regions are aligned within the same expression unit with other proteins or peptides having desired functions, such as for purification or immunodetection purposes (*e.g.*, proteins that may be purified by affinity chromatography and enzyme label coding regions, respectively).
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 Recombinant vectors form further aspects of the present invention. Particularly useful vectors are contemplated to be those vectors in which the coding portion of the DNA segment, whether encoding a full length protein or smaller peptide, is positioned under the
25 control of a promoter. The promoter may be in the form of the promoter that is naturally associated with a LTBP-2 or LTBP-3 gene, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment or exon, for example, using recombinant cloning and/or PCR™ technology, in connection with the compositions disclosed herein.

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5 10 15
In other embodiments, it is contemplated that certain advantages will be gained by positioning the coding DNA segment under the control of a recombinant, or heterologous, promoter. As used herein, a recombinant or heterologous promoter is intended to refer to a promoter that is not normally associated with an LTBP-2 or LTBP-3 gene in its natural environment. Such promoters may include LTBP-2 or LTBP-3 promoters normally associated with other genes, and/or promoters isolated from any bacterial, viral, eukaryotic, or mammalian cell. Naturally, it will be important to employ a promoter that effectively directs the expression of the DNA segment in the cell type, organism, or even animal, chosen for expression. The use of promoter and cell type combinations for protein expression is generally known to those of skill in the art of molecular biology, for example, see Sambrook *et al.*, 1989. The promoters employed may be constitutive, or inducible, and can be used under the appropriate conditions to direct high level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins or peptides. Appropriate promoter systems contemplated for use in high-level expression include, but are not limited to, the *Pichia* expression vector system (Pharmacia LKB Biotechnology).

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In connection with expression embodiments to prepare recombinant LTBP-2 or LTBP-3 proteins and peptides, it is contemplated that longer DNA segments will most often be used, with DNA segments encoding the entire LTBP-2 or LTBP-3 protein or functional domains, subunits, *etc.* being most preferred. However, it will be appreciated that the use of shorter DNA segments to direct the expression of LTBP-2 or LTBP-3 peptides or epitopic core regions, such as may be used to generate anti- LTBP-2 or LTBP-3 antibodies, also falls within the scope of the invention. DNA segments that encode peptide antigens from about 15 to about 50 amino acids in length, or more preferably, from about 15 to about 30 amino acids in length are contemplated to be particularly useful.

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The LTBP-2 or LTBP-3 gene and DNA segments may also be used in connection with somatic expression in an animal or in the creation of a transgenic animal. Again, in such embodiments, the use of a recombinant vector that directs the expression of the full

length or active LTBP-2 or LTBP-3 protein is particularly contemplated.

In addition to their use in directing the expression of the LTBP-2 or LTBP-3 protein, the nucleic acid sequences disclosed herein also have a variety of other uses. For example, they also have utility as probes or primers in nucleic acid hybridization embodiments. As such, it is contemplated that nucleic acid segments that comprise a sequence region that consists of at least a 14 nucleotide long contiguous sequence that has the same sequence as, or is complementary to, a 14 nucleotide long contiguous sequence of SEQ ID NO:1 or SEQ ID NO:3 will find particular utility. Longer contiguous identical or complementary sequences, *e.g.*, those of about 20, 30, 40, 50, 100, 200, 500, 1000 (including all intermediate lengths) and even up to full length sequences will also be of use in certain embodiments.

The ability of such nucleic acid probes to specifically hybridize to LTBP-2 or LTBP-3-encoding sequences will enable them to be of use in detecting the presence of complementary sequences in a given sample. However, other uses are envisioned, including the use of the sequence information for the preparation of mutant species primers, or primers for use in preparing other genetic constructions.

Nucleic acid molecules having sequence regions consisting of contiguous nucleotide stretches of 10-14, 15-20, 30, 50, or even of 100-200 nucleotides or so, identical or complementary to SEQ ID NO:1 or SEQ ID NO:3, are particularly contemplated as hybridization probes for use in, *e.g.*, Southern and Northern blotting. This would allow LTBP-2 or LTBP-3 structural or regulatory genes to be analyzed, both in diverse cell types and also in various mammalian cells. The total size of fragment, as well as the size of the complementary stretch(es), will ultimately depend on the intended use or application of the particular nucleic acid segment. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the contiguous complementary region may be varied, such as between about 10-14 and about 100 nucleotides, but larger contiguous complementarity stretches may be used, according to the length complementary sequences

one wishes to detect.

The use of a hybridization probe of about 10-14 nucleotides in length allows the formation of a duplex molecule that is both stable and selective. Molecules having contiguous complementary sequences over stretches greater than 10 bases in length are generally preferred, though, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 15 to 20 contiguous nucleotides, or even longer where desired.

Hybridization probes may be selected from any portion of any of the sequences disclosed herein. All that is required is to review the sequence set forth in SEQ ID NO:1 or SEQ ID NO:3 and to select any continuous portion of the sequence, from about 10-14 nucleotides in length up to and including the full length sequence, that one wishes to utilize as a probe or primer. The choice of probe and primer sequences may be governed by various factors, such as, by way of example only, one may wish to employ primers from towards the termini of the total sequence.

The process of selecting and preparing a nucleic acid segment that includes a contiguous sequence from within SEQ ID NO:1 or SEQ ID NO:3 may alternatively be described as preparing a nucleic acid fragment. Of course, fragments may also be obtained by other techniques such as, *e.g.*, by mechanical shearing or by restriction enzyme digestion. Small nucleic acid segments or fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer. Also, fragments may be obtained by application of nucleic acid reproduction technology, such as the PCR™ technology of U.S. Patent 4,603,102 (incorporated herein by reference), by introducing selected sequences into recombinant vectors for recombinant production, and by other recombinant DNA techniques generally known to those of skill in the art of molecular biology.

Accordingly, the nucleotide sequences of the invention may be used for their ability to selectively form duplex molecules with complementary stretches of LTBP-2 or LTBP-3 gene or cDNA fragments. Depending on the application envisioned, one will desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of probe towards target sequence. For applications requiring high selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids, *e.g.*, one will select relatively low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of 50°C to 70°C. Such selective conditions tolerate little, if any, mismatch between the probe and the template or target strand, and would be particularly suitable for isolating LTBP-2 or LTBP-3 genes.

Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template or where one seeks to isolate LTBP-2 or LTBP-2 or LTBP-3-encoding sequences from related species, functional equivalents, or the like, less stringent hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ conditions such as about 0.15 M to about 0.9 M salt, at temperatures ranging from 20°C to 55°C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

In certain embodiments, it will be advantageous to employ nucleic acid sequences of the present invention in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including fluorescent, radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal. In preferred embodiments, one will likely desire to employ a fluorescent label or an enzyme tag, such as urease, alkaline phosphatase

or peroxidase, instead of radioactive or other environmental undesirable reagents. In the case of enzyme tags, colorimetric indicator substrates are known that can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with complementary nucleic acid-containing samples.

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In general, it is envisioned that the hybridization probes described herein will be useful both as reagents in solution hybridization as well as in embodiments employing a solid phase. In embodiments involving a solid phase, the test DNA (or RNA) is adsorbed or otherwise affixed to a selected matrix or surface. This fixed, single-stranded nucleic acid is then subjected to specific hybridization with selected probes under desired conditions. The selected conditions will depend on the particular circumstances based on the particular criteria required (depending, for example, on the G+C content, type of target nucleic acid, source of nucleic acid, size of hybridization probe, *etc.*). Following washing of the hybridized surface so as to remove nonspecifically bound probe molecules, specific hybridization is detected, or even quantitated, by means of the label.

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BRIEF DESCRIPTION OF THE DRAWINGS

The drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

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FIG. 1. Overlapping murine cDNA clones representing the LTBP-like (LTBP-2 or LTBP-3) sequence. A partial representation of restriction sites is shown. N, *NcoI*; P, *PvuII*; R, *RsaII*; B, *BamHI*; H, *HindIII*. The numbering system at the bottom assumes that the "A" of the initiator Met codon is nt #1.

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FIG. 2A. A schematic showing the structure of the murine fibrillin-1 gene product.

Structural domains are shown below the diagram. Symbols designating various structural elements are defined in the legend to FIG. 2B.

FIG. 2B. A schematic showing the structure of the murine LTBP-like (LTBP-2 or LTBP-3) molecule. Domains #1-5 are denoted below the diagram. Symbols designate the following structural elements: EGF-CB repeats: open rectangles; TGF-bp repeats: open ovals; Fib motif: open circle; TGF-bp-like repeat: patterned oval; cysteine-rich sequences: patterned rectangles; proline/glycine-rich region: thick curved line, domain #2; proline-rich region, thick curved line, domain #3. Note that symbols designating the signal peptide have been deleted for simplicity. Additionally, the schematic assumes that EGF-like and EGF-CB repeats may extend for several amino acids beyond the C₆ position.

FIG. 2C. A schematic showing the structure of human LTBP-1. Domains #1-5 are denoted below the diagram. The symbols designating the structural elements are defined in the legend to FIG. 2B.

FIG. 3A, FIG. 3B, FIG. 3C, FIG. 3D, FIG. 3E and FIG. 3F. Overview of expression of the new LTBP-like (LTBP-2 or LTBP-3) gene during murine development as determined by tissue *in situ* hybridization. FIG. 3A, FIG. 3B, FIG. 3C, FIG. 3D, FIG. 3E and FIG. 3F are autoradiograms made by direct exposure of tissue sections to film after hybridization with radiolabeled probes. Identical conditions were maintained throughout autoradiography and photography, thereby allowing a comparison of the overall strength of hybridization in all tissue sections. The transcript is expressed in connective tissue, mesenchyme, liver, heart and CNS.

FIG. 3A. Day 8.5-9.0; sections contain embryos surrounded by intact membranes, uterine tissues, and the placental disk, cut in random planes; anti-sense probe.

FIG. 3B. Day 8.5-9.0; sections contain embryos surrounded by intact membranes, uterine tissues, and the placental disk, cut in random planes; sense probe.

FIG. 3C. Day 13.5; sections contain isolated whole embryos sectioned in the sagittal plane near or about the mid-line; anti-sense probe.

FIG. 3D. Day 13.5; sections contain isolated whole embryos sectioned in the sagittal plane near or about the mid-line; sense probe.

FIG. 3E. Day 16.5; sections contain isolated whole embryos sectioned in the sagittal plane near or about the mid-line, anti-sense probe.

FIG. 3F. Day 16.5; sections contain isolated whole embryos sectioned in the sagittal plane near or about the mid-line, sense probe.

FIG. 4. Time-dependent expression of the LTBP-3 gene by MC3T3-E1 cells. mRNA preparation and Northern blotting were performed as described in Example XIV. Equal aliquots of total RNA as determined by UV spectroscopy were loaded in each lane of the Northern gel. As demonstrated by UV spectroscopy were loaded in each lane of the Northern gel. As demonstrated by methylene blue staining (Sambrook *et al.*, 1989), equal amounts of RNA were transferred to the nylon membrane. The results demonstrate a clear, strong peak in LTBP-3 gene expression by 14 days in culture. Weaker signals denoting LTBP-3 gene expression also can be observed after 5 days and 28 days in culture.

FIG. 5. Antisera #274 specifically binds LTBP-3 epitopes. Transfection of 293T cells with a full length mouse LTBP-3 expression plasmid followed by radiolabeling, preparation of medium sample, immunoprecipitation, and 4-18% gradient SDS-PAGE were performed as described in Example XIV. The figure presents a SDS-PAGE autoradiogram of medium samples following a 2 day exposure to film. Lane assignments are as follows: Lane 1, radiolabeled 293T medium (prior to transfection) immunoprecipitated with preimmune serum; Land 2, radiolabeled 293T medium (prior to transfection) immunoprecipitated with antibody #274; Lane 3, radiolabeled 393T medium (following

transfection and preincubation with 10 μ g of LTBP-3 synthetic peptide cocktail) immunoprecipitated with antibody #274; and Lane 4, radiolabeled 293T medium (following transfection) immunoprecipitated with antibody #274. As indicated by the bar, the full length LTBP-3 molecule migrated at 180-190 kDa.

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FIG. 6. Co-immunoprecipitation of LTBP-3 and TGR- β 1 produced by MC3T3-E1 cells. Aliquots ($\sim 10^6$ incorporated CPM) of radiolabeled media produced by MC3T3-E1 cells after 7 days in culture were immunoprecipitated as described in Example XIV. Bars indicate the position of cold molecular weight standards used to estimate molecular weight (Rainbow mix, Amersham). Immunoprecipitates were separated using 4%-18% gradient SDS-PAGE and reducing conditions. The figure shows a negative control lane 1 consisting of MC3T3-E1 medium immunoprecipitated with anti-LTBP-3 antibody #274. Western blotting was performed using the lower portion of the gradient gel and a commercially available antibody to TGF- β 1 (Santa Cruz Biotechnology, Inc.). Antibody staining was detected using commercially available reagents and protocols (ECL Western Blotting Reagent, Amersham). MC3T3-E1 medium was immunoprecipitated with anti-LTBP-2 antibody #274.

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FIG. 7. DNA sequence of the murine LTBP-2 gene (SEQ ID NO:1).

FIG. 8. Amino acid sequence of the murine LTBP-2 gene product (SEQ ID NO:2).

FIG. 9. DNA sequence of the murine LTBP-3 gene (SEQ ID NO:3).

FIG. 10. Amino acid sequence of the murine LTBP-3 gene product (SEQ ID NO:4).

FIG. 11. Mouse *ltbp-2* cDNA Clones. The schematic figure presents overlapping mouse cDNA clones representing the mouse *ltbp-2* coding sequence. A partial representation of the restriction sites is shown. A, *AvrII*; N, *NaeI*; Sa, *ScaII*; X, *XhoI*; B, *BamHI*; C, *ClaI*;

and E, *EcoRI*.

FIG. 12A. *ltbp-2* Gene Expression in the Developing Mouse Skeleton. Shown is an overview of the of the *ltbp-2* gene during mouse development, as determine by tissue in situ hybridization. The figure presents an autoradiogram made by direct exposure of tissue sections to film after hybridization with radiolabeled probes, but before dipping slides in radiographic emulsion. Day 16.5 p.c. sections contain whole embryos sectioned in the mid-sagittal plane. Identical conditions were maintained throughout autoradiography and photography, making it possible to compare the overall strength of hybridization with antisense (top) and sense (bottom) probes. 1 cm = 20 μ m.

FIG. 12B. *ltbp-2* Gene Expression in the Developing Mouse Skeleton. Shown is a selected brightfield microscopic view of mouse *ltbp-2* gene expression in cartilage of day 16.5 p.c. mouse embryos. Photograph was taken from tissue sections following a two week exposure to photographic emulsion. 1 cm = 20 μ m.

FIG. 12C. *ltbp-2* Gene Expression in the Developing Mouse Skeleton. Shown is a selected darkfield microscopic view of mouse *ltbp-2* gene expression in cartilage of day 16.5 p.c. mouse embryos. Photograph was taken from tissue sections following a two week exposure to photographic emulsion. In all darkfield photographs red blood cell and other plasma membranes give a faint white signal that contributes to the background of the study. 1 cm = 20 μ m.

FIG. 13. Co-transfection of 293T Cells With pLTBP-3fl and pTGF- β 1. Immunoprecipitation of LTBP-3 and TGF- β 1 produced by 293T cells following transient transfection and radiolabeling. Aliquots ($\sim 10^6$ incorporated CPM) of radiolabeled media were immunoprecipitated and separated using 4%-18% gradient SDS-PAGE and either reducing or nonreducing conditions as described (Yin et al., 1995). Bars on the left indicate the position of cold standards used to estimate molecular weight: 200, 97.4, 69, 46, 30,

21.5 and 14.3 kDa (Rainbow mix, Amersham). Lane assignments are as follows: Lane 1, 293T cell medium proteins immunoprecipitated with 274 antibody (separated under reducing conditions) after co-transfection with pLTBP-3fl and pTGF- β 1; Lane 2, 293T cell medium proteins immunoprecipitated with 274 antibody (separated under nonreducing conditions) after co-transfection with pLTBP-3fl and pTGF- β 1; Lane 3, untransfected 293T cell medium proteins immunoprecipitated with 274 antibody (separated under reducing conditions); Lane 4, 293T cell medium proteins immunoprecipitated with 274 antibody (separated under reducing conditions) after transfection with pLTBP-3fl; Lane 5, 293T cell medium proteins immunoprecipitated with 40091 antibody (separated under reducing conditions) after transfection with pTGF- β 1; Lane 6, 293T cell medium proteins immunoprecipitated with 40091 antibody (separated under nonreducing conditions) after transfection with pTGF- β 1; and Lane 7, 293T cell medium proteins immunoprecipitated with 40091 antibody (separated under reducing conditions) after co-transfection with pLTBP-3fl and pTGF- β 1. Note that the signal is weakest in lanes 5-7, in which proteins were immunoprecipitated using the 40091 antibody, reflecting the weaker affinity of the 40091 antiserum.

DESCRIPTION OF THE PREFERRED EMBODIMENT

1. Applications of Bone Repair Technology to Human Treatment

The following is a brief discussion of four human conditions to exemplify the variety of diseases and disorders that would benefit from the development of new technology to improve bone repair and healing processes. In addition to the following, several other conditions, such as, for example, vitamin D deficiency; wound healing in general; soft skeletal tissue repair; and cartilage and tendon repair and regeneration, may also benefit from technology concerning the stimulation of bone progenitor cells.

The first example is the otherwise healthy individual who suffers a fracture. Often, clinical bone fracture is treated by casting to alleviate pain and allow natural repair mechanisms to repair the wound. While there has been progress in the treatment of fracture

in recent times, even without considering the various complications that may arise in treating fractured bones, any new procedures to increase bone healing in normal circumstances would represent a great advance.

5 A second example which may benefit from new treatment methods is osteogenesis imperfecta (OI). OI encompasses various inherited connective tissue diseases that involve bone and soft connective tissue fragility in humans (Byers and Steiner, 1992; Prockop, 1990). About one child per 5,000-20,000 born is affected with OI and the disease is associated with significant morbidity throughout life. A certain number of deaths also occur,
10 resulting in part from the high propensity for bone fracture and the deformation of abnormal bone after fracture repair (OI types II-IV; Bonadio and Goldstein, 1993). The relevant issue here is quality of life; clearly, the lives of affected individuals would be improved by the development of new therapies designed to stimulate and strengthen the fracture repair process.

15 OI type I is a mild disorder characterized by bone fracture without deformity, blue sclerae, normal or near normal stature, and autosomal dominant inheritance (Bonadio and Goldstein, 1993). Osteopenia is associated with an increased rate of lone bone fracture upon ambulation (the fracture frequency decreases dramatically at puberty and during young adult life, but increases once again in late middle age). Hearing loss, which often begins in the second or third decade, is a feature of this disease in about half the families and can progress despite the general decline in fracture frequency. Dentinogenesis imperfecta is observed in a subset of individuals.

25 In contrast, OI types II-VI represent a spectrum of more severe disorders associated with a shortened life-span. OI type II, the perinatal lethal form, is characterized by short stature, a soft calvarium, blue sclerae, fragile skin, a small chest, floppy appearing lower extremities (due to external rotation and abduction of the femurs), fragile tendons and ligaments, bone fracture with severe deformity, and death in the perinatal period due to
30 respiratory insufficiency. Radiographic signs of bone weakness include compression of the

femurs, bowing of the tibiae, broad and beaded ribs, and calvarial thinning.

5 OI type III is characterized by short stature, a triangular facies, severe scoliosis, and bone fracture with moderate deformity. Scoliosis can lead to emphysema and a shortened life-span due to respiratory insufficiency. OI type IV is characterized by normal sclerae, bone fracture with mild to moderate deformity, tooth defects, and a natural history that essentially is intermediate between OI type II and OI type I.

10 More than 200 OI mutations have been characterized since 1989 (reviewed in Byers and Steiner, 1992; Prockop, 1990). The vast majority occur in the COL1A1 and COL1A2 genes of type I collagen. Most cases of OI type I appear to result from heterozygous mutations in the COL1A1 gene that decrease collagen production but do not alter primary structure, *i.e.*, heterozygous null mutations affecting COL1A1 expression. Most cases of OI types II-IV result from heterozygous mutations in the COL1A1 and COL1A2 genes that alter the structure of collagen.

15 A third important example is osteoporosis. The term osteoporosis refers to a heterogeneous group of disorders characterized by decreased bone mass and fractures. An estimated 20-25 million people are at increased risk for fracture because of site-specific bone loss. Risk factors for osteoporosis include increasing age, gender (more females), low bone mass, early menopause, race (Caucasians), low calcium intake, reduced physical activity, genetic factors, environmental factors (including cigarette smoking and abuse of alcohol or caffeine), and deficiencies in neuromuscular control that create a propensity to fall.

25 More than a million fractures in the USA each year can be attributed to osteoporosis, and in 1986 alone the treatment of osteoporosis cost an estimated 7-10 billion health care dollars. Demographic trends (*i.e.*, the gradually increasing age of the US population) suggest that these costs may increase 2-3 fold by the year 2020 if a safe and effective treatment is not found. Clearly, osteoporosis is a significant health care problem.

Clinically, osteoporosis is segregated into type I and type II. Type I osteoporosis occurs predominantly in middle aged women and is associated with estrogen loss at the menopause, while osteoporosis type II is associated with advancing age. Much of the morbidity and mortality associated with osteoporosis results from immobilization of elderly patients following fracture.

Current therapies for osteoporosis patients focus on fracture prevention, not fracture repair. This remains an important consideration because of the literature, which clearly states that significant morbidity and mortality are associated with prolonged bed rest in the elderly, particularly those who have suffered hip fractures. Complications of bed rest include blood clots and pneumonia. These complications are recognized and measures are usually taken to avoid them, but these measures hardly represent the best approach to therapy. Thus, the osteoporotic patient population would benefit from new therapies designed to strengthen bone and speed up the fracture repair process, thereby getting these people on their feet before the complications arise.

A fourth example is related to bone reconstruction and, specifically, the ability to reconstruct defects in bone tissue that result from traumatic injury; cancer or cancer surgery; birth defect; a developmental error or heritable disorder; or aging. There is a significant orthopaedic need for more stable total joint implants, and cranial and facial bone are particular targets for this type of reconstructive need. The availability of new implant materials, *e.g.*, titanium, has permitted the repair of relatively large defects. Titanium implants provide excellent temporary stability across bony defects. However, experience has shown that a lack of viable bone bridging the defect can result in exposure of the appliance, infection, structural instability and, ultimately, failure to repair the defect.

Autologous bone grafts are another possible reconstructive modality, but they have several demonstrated disadvantages in that they must be harvested from a donor site such as iliac crest or rib, they usually provide insufficient bone to completely fill the defect, and the bone that does form is sometimes prone to infection and resorption. Partially purified

xenogeneic preparations are not practical for clinical use because microgram quantities are purified from kilograms of bovine bone, making large scale commercial production both costly and impractical. Allografts and demineralized bone preparations are therefore often employed.

Microsurgical transfers of free bone grafts with attached soft tissue and blood vessels can close bony defects with an immediate source of blood supply to the graft. However, these techniques are time consuming, have been shown to produce a great deal of morbidity, and can only be used by specially trained individuals. Furthermore, the bone implant is often limited in quantity and is not readily contoured. In the mandible, for example, the majority of patients cannot wear dental appliances using presently accepted techniques (even after continuity is established), and thus gain little improvement in the ability to masticate. Toriumi *et al.*, have written that, "reconstructive surgeons should have at their disposal a bone substitute that would be reliable, biocompatible, easy to use, and long lasting and that would restore mandibular continuity with little associated morbidity."

In connection with bone reconstruction, specific problem areas for improvement are those concerned with treating large defects, such as created by trauma, birth defects, or particularly, following tumor resection. The success of orthopaedic implants, interfaces and artificial joints could conceivably be improved if the surface of the implant, or a functional part of an implant, were to be coated with a bone stimulatory agent. The surface of implants could be coated with one or more appropriate materials in order to promote a more effective interaction with the biological site surrounding the implant and, ideally, to promote tissue repair.

2. Bone Repair

Bone tissue is known to have the capacity for repair and regeneration and there is a certain understanding of the cellular and molecular basis of these processes. The initiation of new bone formation involves the commitment, clonal expansion, and differentiation of

progenitor cells. Once initiated, bone formation is promoted by a variety of polypeptide growth factors. Newly formed bone is then maintained by a series of local and systemic growth and differentiation factors.

5 The concept of specific bone growth-promoting agents is derived from the work of Huggins and Urist. Huggins *et al.*, 1936, demonstrated that autologous transplantation of canine incisor tooth to skeletal muscle resulted in local new bone formation (Huggins *et al.*, 1936). Urist and colleagues reported that demineralized lyophilized bone segments induced bone formation (Urist, 1965; Urist *et al.*, 1983), a process that involved macrophage
10 chemotaxis; the recruitment of progenitor cells; the formation of granulation tissue, cartilage, and bone; bone remodeling; and marrow differentiation. The initiation of cartilage and bone formation in an extraskeletal site, a process referred to as osteoinduction, has permitted the unequivocal identification of initiators of bone morphogenesis (Urist, 1965; Urist *et al.*, 1983; Sampath *et al.*, 1984; Wang *et al.*, 1990; Cunningham *et al.*, 1992).

15 Significant progress has now been made in characterizing the biological agents elaborated by active bone tissue during growth and natural bone healing. Demineralized bone matrix is highly insoluble; Sampath and Reddi (1981) showed that only 3% of the proteins can be extracted using strong combinations of denaturants and detergents. They also
20 showed that the unfractionated demineralized bone extract will initiate bone morphogenesis, a critical observation that led to the purification of "osteoinductive" molecules. Families of proteinaceous osteoinductive factors have now been purified and characterized. They have been variously referred to in the literature as bone morphogenetic or morphogenic proteins (BMPs), osteogenic bone inductive proteins or osteogenic proteins (OPs).

25 3. Bone Repair and Growth Factors and Cytokines

 Following their initial purification, several bone morphogenetic protein genes have now been cloned using molecular techniques (Wozney *et al.*, 1988; Rosen *et al.*, 1989;

summarized in Alper, 1994). This work has established BMPs as members of the transforming growth factor- β (TGF- β) superfamily based on DNA sequence homologies. Other TGF molecules have also been shown to participate in new bone formation, and TGF- β is regarded as a complex multifunctional regulator of osteoblast function (Centrella *et al.*, 1988; Carrington *et al.*, 1988; Seitz *et al.*, 1992). Indeed, the family of transforming growth factors (TGF- β 1, TGF- β 2, and TGF- β 3) has been proposed as potentially useful in the treatment of bone disease (U.S. Patent 5,125,978, incorporated herein by reference).

Transforming growth factors (TGFs) have a central role in regulating tissue healing by affecting cell proliferation, gene expression, and matrix protein synthesis (Roberts and Sporn, 1989). While not necessarily a direct effect, evidence has been provided that TGF- β 1 and TGF- β 2 can initiate both chondrogenesis and osteogenesis (Joyce *et al.*, 1990; Izumi *et al.*, 1992; Jingushi *et al.*, 1992). In these studies new cartilage and bone formation appeared to be dose dependent (*i.e.*, dependent on the local growth factor concentration). The data also suggested that TGF- β 1 and TGF- β 2 stimulated cell differentiation by a similar mechanism, even though they differed in terms of the ultimate amount of new cartilage and bone that was formed.

Other growth factors/hormones besides TGF and BMP may influence new bone formation following fracture. Bolander and colleagues injected recombinant acidic fibroblast growth factor into a rat fracture site (Jingushi *et al.*, 1990). The major effect of multiple high doses (1.0 mg/50 ml) was a significant increase in cartilage tissue in the fracture gap, while lower doses had no effect. These investigators also used the reverse transcriptase-polymerase chain reaction (PCR[™]) technique to demonstrate expression of estrogen receptor transcripts in callus tissue (Boden *et al.*, 1989). These results suggested a role for estrogen in normal fracture repair.

Horowitz and colleagues have shown that activated osteoblasts will synthesize the cytokine, macrophage colony stimulating factor (Horowitz *et al.*, 1989). The osteotropic

agents used in this study included lipopolysaccharide, PTH1-84, PTH1-34, vitamin D and all-trans retinoic acid. This observation has led to the suggestion that osteoblast activation following fracture may lead to the production of cytokines that regulate both hematopoiesis and new bone formation. Various other proteins and polypeptides that have been found to be expressed at high levels in osteogenic cells, such as, *e.g.*, the polypeptide designated Vgr-1 (Lyons *et al.*, 1989), also have potential for use in connection with the present invention.

4. Protein Administration and Bone Repair

Several studies have been conducted in which preparations of protein growth factors, including BMPs, have been administered to animals in an effort to stimulate bone growth. The results of four such exemplary studies are described below.

Toriumi *et al.*, studied the effect of recombinant BMP-2 on the repair of surgically created defects in the mandible of adult dogs (Toriumi *et al.*, 1991). Twenty-six adult hounds were segregated into three groups following the creation of a 3 cm full thickness mandibular defect: 12 animals received test implants composed of inactive dog bone matrix carrier and human BMP-2, 10 animals received control implants composed of carrier without BMP-2, and BMP-4 animals received no implant. The dogs were euthanized at 2.5-6 months, and the reconstructed segments were analyzed by radiography, histology, histomorphometry, and biomechanical testing. Animals that received test implants were euthanized after 2.5 months because of the presence of well mineralized bone bridging the defect. The new bone allowed these animals to chew a solid diet, and the average bending strength of reconstructed mandibles was 27% of normal ('normal' in this case represents the unoperated, contralateral hemimandible). In contrast, the implants in the other two groups were non-functional even after 6 months and showed minimal bone formation.

Yasko *et al.*, published a related study in which the effect of BMP-2 on the repair of segmental defects in the rat femur was examined (Yasko *et al.*, 1992). The study design

included a group that received a dose of 1.4 mg of BMP-2, another group that received 11.0 mg of BMP-2, and a control group that received carrier matrix alone. Endochondral bone formation was observed in both groups of animals that received BMP-2. As demonstrated by radiography, histology, and whole bone (torsion) tests of mechanical integrity, the larger dose resulted in functional repair of the 5-mm defect beginning 4.5 weeks after surgery. The lower dose resulted in radiographic and histological evidence of new bone formation, but functional union was not observed even after 9 weeks post surgery. There was also no evidence of bone formation in control animals at this time.

Chen *et al.*, showed that a single application of 25-100 mg of recombinant TGF- β 1 adjacent to cartilage induced endochondral bone formation in the rabbit ear full thickness skin wounds (Chen *et al.*, 1991). Bone formation began 21 days following the creation of the wound and reached a peak at day 42, as demonstrated by morphological methods. Active bone remodeling was observed beyond this point.

In a related study, Beck *et al.*, demonstrated that a single application of TGF- β 1 in a 3% methylcellulose gel was able to repair surgically induced large skull defects that otherwise heal by fibrous connective tissue and never form bone (Beck *et al.*, 1991). Bony closure was achieved within 28 days of the application of 200 mg of TGF- β 1 and the rate of healing was shown to be dose dependent.

Studies such as those described above have thus established that exogenous growth factors can be used to stimulate new bone formation/repair/regeneration *in vivo*. Certain U.S. Patents also concern methods for treating bone defects or inducing bone formation. For example, U.S. Patent 4,877,864 relates to the administration of a therapeutic composition of bone inductive protein to treat cartilage and/or bone defects; U.S. Patent 5,108,753 concerns the use of a device containing a pure osteogenic protein to induce endochondral bone formation and for use in periodontal, dental or craniofacial reconstructive procedures.

5. Recombinant Expression

The use of recombinant expression systems in the preparation of LTBP-2 and LTBP-3 polypeptides is particularly contemplated. To express a recombinant LTBP-2 and LTBP-3 polypeptides, whether mutant or wild-type, in accordance with the present invention one would prepare an expression vector that comprises an LTBP-2- or LTBP-3-encoding nucleic acid segment under the control of one or more promoters. The "upstream" promoters stimulate transcription of the DNA and promote expression of the encoded recombinant protein. This is the meaning of "recombinant expression" in this context.

Many standard techniques are available to construct expression vectors containing the appropriate nucleic acids and transcriptional/translational control sequences in order to achieve protein or peptide expression in a variety of host-expression systems. Cell types available for expression include, but are not limited to, bacteria, such as *E. coli* and *B. subtilis* transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors.

Certain examples of prokaryotic hosts are *E. coli* strain RR1, *E. coli* LE392, *E. coli* B, *E. coli* X 1776 (ATCC No. 31537) as well as *E. coli* W3110 (F-, lambda-, prototrophic, ATCC No. 27325); bacilli such as *Bacillus subtilis*; and other enterobacteriaceae such as *Salmonella typhimurium*, *Serratia marcescens*, and various *Pseudomonas* species.

In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is often transformed using pBR322, a plasmid derived from an *E. coli* species. pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying

transformed cells. The pBR plasmid, or other microbial plasmid or phage must also contain, or be modified to contain, promoters which can be used by the microbial organism for expression of its own proteins.

5 In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as transforming vectors in connection with these hosts. For example, the phage lambda GEM-11™ may be utilized in making a recombinant phage vector which can be used to transform host cells, such as *E. coli* LE392. Further useful vectors include pIN vectors (Inouye *et al.*, 1985); and pGEX vectors, for use
10 in generating glutathione S-transferase (GST) soluble fusion proteins for later purification and separation or cleavage.

Promoters that are most commonly used in recombinant DNA construction include the β -lactamase (penicillinase), lactose and tryptophan (*trp*) promoter systems. While these are the most commonly used, other microbial promoters have been discovered and utilized, and details concerning their nucleotide sequences have been published, enabling those of skill in the art to ligate them functionally with plasmid vectors.

For expression in *Saccharomyces*, the plasmid YRp7, for example, is commonly used
20 (Stinchcomb *et al.*, 1979; Kingsman *et al.*, 1979; Tschemper *et al.*, 1980). This plasmid already contains the *trpL* gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, 1977). The presence of the *trpL* lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of
25 tryptophan.

Suitable promoting sequences in yeast vectors include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, 1980) or other glycolytic enzymes (Hess *et al.*, 1968; Holland *et al.*, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase,

hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. In constructing suitable expression plasmids, the termination sequences associated with these genes are also ligated into the expression vector 3' of the sequence desired to be expressed to provide polyadenylation of the mRNA and termination.

Other suitable promoters, which have the additional advantage of transcription controlled by growth conditions, include the promoter region for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, and the aforementioned glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization.

In addition to micro-organisms, cultures of cells derived from multicellular organisms may also be used as hosts. In principle, any such cell culture is workable, whether from vertebrate or invertebrate culture. In addition to mammalian cells, these include insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus); and plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing one or more LTBP-2- or LTBP-3- encoding DNA sequences.

In a useful insect system, *Autographa californica* nuclear polyhidrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The LTBP coding sequences are cloned into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the coding sequences results in the inactivation of the polyhedrin gene and production of non-occluded recombinant virus (*i.e.*, virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is

expressed (*e.g.*, U.S. Patent No. 4,215,051 issued to Smith).

5 Examples of useful mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, W138, BHK, COS-7, 293, HepG2, 3T3, RIN and MDCK cell lines. In addition, a host cell strain may be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein, particularly with respect to the ability of LTBP-2 and LTBP-3 polypeptides to bind to TGF β proteins.

10 Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cells lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells may also be used if desired, with a cell that allows for high-level expression of LTBP-2 and LTBP-3 polypeptides being preferred.

15 Expression vectors for use in mammalian such cells ordinarily include an origin of replication (as necessary), a promoter located in front of the gene to be expressed, along with any necessary ribosome binding sites, RNA splice sites, polyadenylation site, and transcriptional terminator sequences. The origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral (*e.g.*, Polyoma, Adeno, VSV, BPV) source, or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter is often sufficient.

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25 The promoters may be derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). Further, it is also possible, and may be desirable, to utilize promoter or control sequences normally associated with the desired LTBP-encoding

gene sequence, provided such control sequences are compatible with the host cell systems.

A number of viral based expression systems may be utilized, for example, commonly used promoters are derived from polyoma, Adenovirus 2, and most frequently Simian Virus 40 (SV40). The early and late promoters of SV40 virus are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication. Smaller or larger SV40 fragments may also be used, provided there is included the approximately 250 bp sequence extending from the Hind III site toward the BglII site located in the viral origin of replication.

In cases where an adenovirus is used as an expression vector, the coding sequences may be ligated to an adenovirus transcription/ translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the LTBP polypeptides in infected hosts.

Specific initiation signals may also be required for efficient translation of LTBP-2 and LTBP-3 coding sequences. These signals include the ATG initiation codon and adjacent sequences. Exogenous translational control signals, including the ATG initiation codon, may additionally need to be provided. One of ordinary skill in the art would readily be capable of determining this and providing the necessary signals. It is well known that the initiation codon must be in-frame (or in-phase) with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators (Bittner *et al.*, 1987).

For long-term, high-yield production of recombinant LTBP-2 or LTBP-3 proteins, stable expression is preferred. For example, cell lines that stably express constructs

encoding LTBP-2 or LTBP-3 polypeptides may be engineered. Rather than using expression vectors that contain viral origins of replication, host cells can be transformed with vectors controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, *etc.*), and a selectable marker. Following the introduction of foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines.

A number of selection systems may be used, including, but not limited, to the herpes simplex virus thymidine kinase (Wigler *et al.*, 1977), hypoxanthine-guanine phosphoribosyltransferase (Szybalska *et al.*, 1962) and adenine phosphoribosyltransferase genes (Lowy *et al.*, 1980), in tk-, hgp^{rt}- or ap^{rt}- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, that confers resistance to methotrexate (Wigler *et al.*, 1980; O'Hare *et al.*, 1981); gpt, that confers resistance to mycophenolic acid (Mulligan *et al.*, 1981); neo, that confers resistance to the aminoglycoside G-418 (Colberre-Garapin *et al.*, 1981); and hyg^r, that confers resistance to hygromycin (Santerre *et al.*, 1984).

6. Site-Specific Mutagenesis

Site-specific mutagenesis is a technique useful in the preparation of individual peptides, or biologically functional equivalent proteins or peptides, through specific mutagenesis of the underlying DNA. The technique further provides a ready ability to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the DNA. Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well

as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Typically, a primer of about 17 to 25 nucleotides in length is preferred, with about 5 to 10 residues on both sides of the junction of the sequence being altered.

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In general, the technique of site-specific mutagenesis is well known in the art, as exemplified by various publications. As will be appreciated, the technique typically employs a phage vector which exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phage are readily commercially available and their use is generally well known to those skilled in the art. Double stranded plasmids are also routinely employed in site directed mutagenesis which eliminates the step of transferring the gene of interest from a plasmid to a phage.

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In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector or melting apart of two strands of a double stranded vector which includes within its sequence a DNA sequence which encodes the desired osteotropic protein. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically. This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as *E. coli* cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement.

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The preparation of sequence variants of the selected LTBP-2 or LTBP-3 gene using site-directed mutagenesis is provided as a means of producing potentially useful species and is not meant to be limiting as there are other ways in which sequence variants of osteotropic genes may be obtained. For example, recombinant vectors encoding the desired osteotropic

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gene may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants.

7. *In Situ* Hybridization

The following technique describes the detection of mRNA in tissue obtained from the site of bone regeneration. This may be useful for detecting expression of TBP-2 or LTBP-3.

DNA from a plasmid containing the gene for which mRNA is to be detected is linearized, extracted, and precipitated with ethanol. Sense and antisense transcripts are generated from 1 mg template with T3 and T7 polymerases, *e.g.*, in the presence of [³⁵S] UTP at >6 mCi/ml (Amersham Corp., >1200 Ci/mmol) and 1.6 U/ml RNasin (Promega), with the remaining *in vitro* transcription reagents provided in a kit (SureSite, Novagen Inc.). After transcription at 37°C for 1 hour, DNA templates are removed by a 15 minute digestion at 37°C with 0.5 U/ml RNase-free DNase I, extracted, and precipitated with ethanol. Riboprobes are hydrolyzed to an average final length of 150 bp by incubating in 40 mM NaHCO₃, 60 mM Na₂CO₃, 80 mM DTT at 60°C, according to previously determined formula. Hydrolysis is terminated by addition of sodium acetate, pH 6.0, and glacial acetic acid to 0.09 M and 0.005% (v/v), respectively, and the probes are then ethanol precipitated, dissolved in 0.1 M DTT, counted, and stored at -20°C until use.

RNase precautions are taken in all stages of slide preparation. Bouins fixed, paraffin embedded tissue sections are heated to 65°C for 10 minutes, deparaffinized in 3 changes of xylene for 5 minutes, and rehydrated in a descending ethanol series, ending in phosphate-buffered saline (PBS). Slides will be soaked in 0.2 N HCl for 5 min., rinsed in PBS, digested with 0.0002% proteinase K in PBS for 30 minutes at 37°C and rinsed briefly with DEPC-treated water. After equilibrating for 3 minutes in 0.1 M triethanolamine-HCl (TEA-HCl), pH 8.0, sections are acetylated in 0.25% (v/v) acetic anhydride in 0.1 M TEA-HCl for 10 minutes at room temperature, rinsed in PBS, and dehydrated in an ascending ethanol

series. Each section receives 100-200 ml prehybridization solution (0.5 mg/ml denatured RNase-free tRNA (Boehringer-Mannheim), 10 mM DTT, 5 mg/ml denatured, sulfurylated salmon sperm DNA, 50% formamide, 10% dextran sulfate, 300 mM NaCl, 1X RNase-free Denhardt's solution (made with RNase-free bovine serum albumin, Sigma), 10 mM Tris-HCl, pH 7.4, 1 mM EDTA) and then incubate on a 50°C slide warmer in a humidified enclosure for 2 hours. The sulfurylated salmon-sperm DNA blocking reagent is used in both prehybridization and hybridization solutions to help reduce nonspecific binding to tissue by ³⁵S groups on the probe. It is prepared by labeling RNase-free salmon sperm DNA (Sigma) with non-radioactive α -thio-dCTP and α -thio-dATP (Amersham) in a standard random oligonucleotide-primed DNA labeling reaction. Excess prehybridization solution is removed with a brief rinse in 4X SSC before application of probe.

Riboprobes, fresh tRNA and sulfurylated salmon sperm DNA will be denatured for 10 minutes at 70°C, and chilled on ice. Hybridization solution, identical to prehybridization solution except with denatured probe added to 5×10^6 CPM/ml, is applied and slides incubated at 50°C overnight in sealed humidified chambers on a slide warmer. Sense and antisense probes are applied to serial sections. Slides are rinsed 3 times in 4X SSC, washed with 2X SSC, 1 mM DTT for 30 min. at 50°C, digested with RNase A (20 mg/ml RNase A, 0.5 M NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA, pH 8.0) for 30 min. at 37°C, and rinsed briefly with 2X SSC, 1 mM DTT. Three additional washes are performed, each at 50°C for 30 minutes: once in 2X SSC, 50% formamide, 1 mM DTT, and twice in 1X SSC, 0.13% (w/v) sodium pyrophosphate, 1 mM DTT.

Slides are dehydrated in an ascending ethanol series (with supplementation of the dilute ethanols (50% and 70%) with SSC and DTT to 0.1X and 1 mM, respectively). Slides are exposed to X-ray film for 20-60 hours to visualize overall hybridization patterns, dipped in autoradiographic emulsion (Kodak NTB-2, diluted to 50% with 0.3 M ammonium acetate), slowly dried for 2 hours, and exposed (4°C) for periods ranging from 8 days to 8 weeks. After developing emulsion, sections are counter stained with hematoxylin and eosin, dehydrated, and mounted with xylene-based medium. The hybridization signal is visualized

under darkfield microscopy.

The above *in situ* hybridization protocol may be used, for example, in detecting the temporal and spatial pattern of PTH/PTHrP receptor expression. A suitable rat PTH/PTHrP receptor cDNA probe (R15B) is one that consists of a 1810 bp region encoding the full length rat bone PTH/PTHrP receptor (Abou-Samra *et al.*, 1992). The cDNA fragment is subcloned into pDNA 1 (Invitrogen Corp., San Diego, CA) and is cut out using *Xba*I and *Bam*HI. This probe has provided positive signals for northern blot analysis of rat, murine, and human osteoblastic cell lines, rat primary calvarial cells, and murine bone tissue. The pcDNA I plasmid contains a T7 and SP6 promoter that facilitate the generation of cRNA probes for *in situ* hybridization. The full length transcript has been used to detect PTH/PTHrP receptor in sections of bone (Lee *et al.*, 1994). The PTHrP cDNA probe (Yasuda *et al.*, 1989) is a 400 bp subcloned fragment in pBluescript (Stratagene). This probe has been used for *in situ* hybridization, generating an antisense cRNA probe using *Bam*HI cleavage and the T3 primer and a sense cRNA probe using *Eco*RI cleavage and the T7 primer.

8. Monoclonal Antibody Generation

Means for preparing and characterizing antibodies are well known in the art (See, *e.g.*, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988; incorporated herein by reference).

The methods for generating monoclonal antibodies (MAbs) generally begin along the same lines as those for preparing 'polyclonal antibodies. Briefly, a polyclonal antibody is prepared by immunizing an animal with an immunogenic composition in accordance with the present invention and collecting antisera from that immunized animal. A wide range of animal species can be used for the production of antisera. Typically the animal used for production of anti-antisera is a rabbit, a mouse, a rat, a hamster, a guinea pig or a goat.

Because of the relatively large blood volume of rabbits, a rabbit is a preferred choice for production of polyclonal antibodies.

As is well known in the art, a given composition may vary in its immunogenicity. It is often necessary therefore to boost the host immune system, as may be achieved by coupling a peptide or polypeptide immunogen to a carrier. Exemplary and preferred carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other albumins such as ovalbumin, mouse serum albumin or rabbit serum albumin can also be used as carriers. Means for conjugating a polypeptide to a carrier protein are well known in the art and include glutaraldehyde, m-maleimidobencoyl-N-hydroxysuccinimide ester, carbodiimide and bis-biazotized benzidine.

As is also well known in the art, the immunogenicity of a particular immunogen composition can be enhanced by the use of non-specific stimulators of the immune response, known as adjuvants. Exemplary and preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis*), incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

The amount of immunogen composition used in the production of polyclonal antibodies varies upon the nature of the immunogen as well as the animal used for immunization. A variety of routes can be used to administer the immunogen (subcutaneous, intramuscular, intradermal, intravenous and intraperitoneal). The production of polyclonal antibodies may be monitored by sampling blood of the immunized animal at various points following immunization. A second, booster injection, may also be given. The process of boosting and titering is repeated until a suitable titer is achieved. When a desired level of immunogenicity is obtained, the immunized animal can be bled and the serum isolated and stored, and/or the animal can be used to generate MAbs.

MAbs may be readily prepared through use of well-known techniques, such as those exemplified in U.S. Patent 4,196,265, incorporated herein by reference. Typically, this

technique involves immunizing a suitable animal with a selected immunogen composition, e.g., a purified or partially purified LTBP-2 or LTBP-3 protein, polypeptide or peptide. The immunizing composition is administered in a manner effective to stimulate antibody producing cells. Rodents such as mice and rats are preferred animals, however, the use of rabbit, sheep frog cells is also possible. The use of rats may provide certain advantages (Goding, 1986, pp. 60-61), but mice are preferred, with the BALB/c mouse being most preferred as this is most routinely used and generally gives a higher percentage of stable fusions.

Following immunization, somatic cells with the potential for producing antibodies, specifically B lymphocytes (B cells), are selected for use in the MAb generating protocol. These cells may be obtained from biopsied spleens, tonsils or lymph nodes, or from a peripheral blood sample. Spleen cells and peripheral blood cells are preferred, the former because they are a rich source of antibody-producing cells that are in the dividing plasmablast stage, and the latter because peripheral blood is easily accessible. Often, a panel of animals will have been immunized and the spleen of animal with the highest antibody titer will be removed and the spleen lymphocytes obtained by homogenizing the spleen with a syringe. Typically, a spleen from an immunized mouse contains approximately 5×10^7 to 2×10^8 lymphocytes.

The antibody-producing B lymphocytes from the immunized animal are then fused with cells of an immortal myeloma cell, generally one of the same species as the animal that was immunized. Myeloma cell lines suited for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas).

Any one of a number of myeloma cells may be used, as are known to those of skill in the art (Goding, pp. 65-66, 1986; Campbell, pp. 75-83, 1984). For example, where the immunized animal is a mouse, one may use P3-X63/Ag8, X63-Ag8.653, NS1/1.Ag 4 1,

Sp210-Ag14, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XX0 Bul; for rats, one may use R210.RCY3, Y3-Ag 1.2.3, IR983F and 4B210; and U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6 are all useful in connection with human cell fusions.

5 One preferred murine myeloma cell is the NS-1 myeloma cell line (also termed P3-NS-1-Ag4-1), which is readily available from the NIGMS Human Genetic Mutant Cell Repository by requesting cell line repository number GM3573. Another mouse myeloma cell line that may be used is the 8-azaguanine-resistant mouse murine myeloma SP2/0 non-producer cell line.

10 Methods for generating hybrids of antibody-producing spleen or lymph node cells and myeloma cells usually comprise mixing somatic cells with myeloma cells in a 2:1 proportion, though the proportion may vary from about 20:1 to about 1:1, respectively, in the presence of an agent or agents (chemical or electrical) that promote the fusion of cell membranes. Fusion methods using Sendai virus have been described by Kohler and Milstein (1975; 15 1976), and those using polyethylene glycol (PEG), such as 37% (v/v) PEG, by Gefter *et al.* (1977). The use of electrically induced fusion methods is also appropriate (Goding pp. 71-74, 1986).

20 Fusion procedures usually produce viable hybrids at low frequencies, about 1×10^{-6} to 1×10^{-8} . However, this does not pose a problem, as the viable, fused hybrids are differentiated from the parental, unfused cells (particularly the unfused myeloma cells that would normally continue to divide indefinitely) by culturing in a selective medium. The selective medium is generally one that contains an agent that blocks the *de novo* synthesis of 25 nucleotides in the tissue culture media. Exemplary and preferred agents are aminopterin, methotrexate, and azaserine. Aminopterin and methotrexate block *de novo* synthesis of both purines and pyrimidines, whereas azaserine blocks only purine synthesis. Where aminopterin or methotrexate is used, the media is supplemented with hypoxanthine and thymidine as a source of nucleotides (HAT medium). Where azaserine is used, the media is supplemented 30 with hypoxanthine.

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The preferred selection medium is HAT. Only cells capable of operating nucleotide salvage pathways are able to survive in HAT medium. The myeloma cells are defective in key enzymes of the salvage pathway, *e.g.*, hypoxanthine phosphoribosyl transferase (HPRT), and they cannot survive. The B cells can operate this pathway, but they have a limited life span in culture and generally die within about two weeks. Therefore, the only cells that can survive in the selective media are those hybrids formed from myeloma and B cells.

This culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants (after about two to three weeks) for the desired reactivity. The assay should be sensitive, simple and rapid, such as radioimmunoassays, enzyme immunoassays, cytotoxicity assays, plaque assays, dot immunobinding assays, and the like.

The selected hybridomas would then be serially diluted and cloned into individual antibody-producing cell lines, which clones can then be propagated indefinitely to provide MAbs. The cell lines may be exploited for MAb production in two basic ways. A sample of the hybridoma can be injected (often into the peritoneal cavity) into a histocompatible animal of the type that was used to provide the somatic and myeloma cells for the original fusion. The injected animal develops tumors secreting the specific monoclonal antibody produced by the fused cell hybrid. The body fluids of the animal, such as serum or ascites fluid, can then be tapped to provide MAbs in high concentration. The individual cell lines could also be cultured *in vitro*, where the MAbs are naturally secreted into the culture medium from which they can be readily obtained in high concentrations. MAbs produced by either means may be further purified, if desired, using filtration, centrifugation and various chromatographic methods such as HPLC or affinity chromatography.

9. LTBP Structure and Function

The LTBP appear to be modular polypeptides characterized by the presence of

multiple cysteine-rich motifs. Molecular cloning of human *LTBP-1* (Kanzaki *et al.*, 1990) indicates, for example, that the molecule consists of 16 epidermal growth factor-like repeats with the potential to bind calcium (EGF-CB repeats), 3 copies of a unique motif containing 8 cysteine residues, an RGD motif, and an 8 amino acid motif identical to the cell binding domain of the laminin $\beta 2$ chain. EGF-CB repeats may be modified to contain hydroxyaspartic acid and hydroxyasparagine (Stenflo *et al.*, 1987). The genes that code for two LTBP's have been isolated previously. In sharp contrast to those of the prior art, the LTBP-3 sequence of the present invention shares only 40% sequence identity with those of the LTBP-1 sequence (Kanzaki *et al.*, 1990) and the LTBP-2 sequence (Moren *et al.*, 1994).

Unlike the human *LTBP* genes isolated previously which are localized to human chromosome 2, band p12-q22 (*LTBP-1*, Stenman *et al.*, 1994) and human chromosome 14, band q24 (*LTBP-2*, Moren *et al.*, 1994), the *LTBP-3* of the present invention is localized to chromosome 11, band q12.

One aspect of the present invention is the mapping of the murine *LTBP-3* to mouse chromosome 19, band B, a region of conserved synteny with human chromosome 11, band q12.

While the function of LTBP is unknown (*i.e.*, studies with transfected CHO cells indicate that LTBP does not contribute to TGF- β latency), several ideas have been proposed that, when taken together, suggest that LTBP may function as a extracellular structural protein capable of both regulating and targeting TGF- β activity. First, LTBP may regulate the intracellular biosynthesis of latent TGF- β . Cultured erythroleukemia cells efficiently assemble and secrete large latent TGF- β complexes, but they poorly secrete latent TGF- β complexes that lack LTBP-1 (Miyazono *et al.*, 1991; 1992). The retained complexes contain anomalous disulfide bonds, suggesting that, for erythroleukemia cells at least, LTBP contributes to the normal assembly and secretion of TGF- β latent complexes. Second, LTBP may function to target latent TGF- β to specific types of connective tissue. Recent evidence

suggests that the large latent TGF- β complex covalently binds the extracellular matrix via LTBP-1, *i.e.*, LTBP-1 may target the latent TGF- β complex to a site near the cell surface (pericellular matrix) to facilitate the generation of autocrine or paracrine effects (Flaumenhaft *et al.*, 1993; Taipale *et al.*, 1994; Taipale and Keski-Oja, 1992). Third, LTBP may modulate the activation of latent complexes. There is direct evidence that LTBP-1 binds calcium (Colosetti *et al.*, 1993) and that calcium binding induces a structural change that protects LTBP-1 from proteolytic attack. As described above (Lyons *et al.*, 1988; Taipale *et al.*, 1995), latent TGF- β is exposed to protease-rich environments during wound repair and normal development and exposure to these environments leads to the release of mature TGF- β from extracellular storage sites. It is therefore possible that the protease-resistant conformation of LTBP helps ensure TGF- β integrity *in vivo*.

10. LTBP and Skeletal Tissues

Skeletal tissue represents one of the largest known repositories of latent TGF- β (200 $\mu\text{g/kg}$ bone; Seyedin *et al.*, 1986; Seyedin *et al.*, 1987). Moreover, activated TGF- β may stimulate bone formation in developing tissues and may act as a "coupling factor" that coordinates matrix resorption and formation during bone remodeling (Centrella *et al.*, 1991). Finally, activated TGF- β may exert a powerful osteoinductive stimulus following fracture (Joyce *et al.*, 1990; Beck *et al.*, 1993).

It has not yet been determined if TGF- β normally exists in bone as a small or large latent complex. Previous studies (Pfeilschifter *et al.*, 1990; Bonewald *et al.*, 1991) have shown that the major form of TGF- β in conditioned media from bone organ cultures is a 100 kDa latent complex that lacks LTBP-1. Additionally, Dallas *et al.* (Dallas *et al.*, 1994) have demonstrated that cultured MG63, ROS 17/2.8, and UMR-106 cells (derived from osteosarcomas of various types) each secrete two major forms of latent TGF- β 1, namely, a 290 kDa complex that contains LTBP-1 and a 100 kDa complex lacking LTBP-1. A second high molecular weight complex that contained latent TGF- β 2 and LTBP was also identified.

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The presence and relative amount of low and high molecular weight complexes varied with cell type, and TGF- β 1 and LTBP did not appear to be co-expressed in bone cells. These results led the authors to conclude that the 100 kDa latent TGF- β complex is a physiologically important form in bone cells, *i.e.*, LTBP did not appear to be required for the proper and efficient assembly and secretion of small latent complexes.

At the end of their paper, Dallas *et al.* also acknowledged that the production of small versus large latent TGF- β complexes may be associated with specific stages in the maturation of bone cells — *e.g.*, MG63, ROS 17/2.8, and UMR-106 cells are known to express different subsets of mature osteoblast phenotypic markers, which could explain differences in the size of the latent TGF- β complexes produced by the various osteosarcoma cell lines. Along this line, the inventors' laboratory has recently shown that mouse pre-osteoblast MC3T3-E1 cells express the *ltbp-3* gene at the outset of osteoblast differentiation and that the LTBP-3 polypeptide binds TGF- β 1 in MC3T3-E1 conditioned media (Yin *et al.*, 1995). Therefore, MC3T3-E1 cells express large latent TGF- β 1 complexes bearing LTBP-3 precisely at the time of the pre-osteoblast to osteoblast transition (*i.e.*, at ~day 14 in culture, or at the onset of alkaline phosphatase expression).

Extending the MC3T3-E1 data further, the inventors' laboratory has also found that *ltbp-2* and *ltbp-3* are co-expressed with TGF- β in developing mouse skeletal tissues (Yin *et al.*, 1995) and that *ltbp-3* (at least) is expressed at sites of osteotomy repair *in vivo* (Yin *et al.*, 1994).

There is little doubt that TGF- β contributes to the normal structure and function of skeletal tissues. Bone is an abundant source of latent TGF- β , and mature TGF- β contributes to the processes of skeletal morphogenesis, bone remodeling, and bone repair. Consequently, bone must regulate the autocrine and paracrine effects of TGF- β with precision. The inventors' laboratory was the first to clone and map the mouse *ltbp-2* and *ltbp-3* genes, and it has obtained evidence that both genes are expressed during normal

murine skeletal morphogenesis and during bone osteotomy repair. In addition, in MC3T3-E1 pre-osteoblasts TGF- β is synthesized as a homodimer known as the small latent complex that covalently binds LTBP to form large latent complexes. These results suggest for the first time that LTBP facilitates the assembly and secretion of latent TGF- β complexes and targets latent TGF- β to bone matrix. With the availability of the *ltbp-2* and *ltbp-3* genes, the opportunity exists in the inventors' laboratory to gain further insight into LTBP structure and function and, in turn, the mechanism by which latent TGF- β complexes can be targeted to bone matrix and cells in a controlled manner.

11. Detection of LTBP-encoding DNA Segments

The amount of an LTBP-2 or LTBP-3-encoding DNA segment present within a biological sample, such as blood, serum or PBMC sample, may be determined by means of a molecular biological assay to determine the level of a nucleic acid that encodes such an LTBP-2 or LTBP-3 polypeptide, or by means of an immunoassay to determine the level of the polypeptide itself.

In a molecular biological method for detecting a cell that produces LTBP-2 or LTBP-3, one would obtain nucleic acids from one or more cells and analyze the nucleic acids to identify a nucleic acid segment that encodes LTBP-2 or LTBP-3. Such nucleic acids may be identified by length, where an appropriate assay would be a PCR™-based assay resulting in the identification of an LTBP-2 or LTBP-3-encoding mRNA transcript. Alternatively, the nucleic acid segment may be identified by sequence, which method generally includes identifying a transcript with a sequence of the present invention *e.g.*, by Northern or Southern blotting using a discriminating probe prepared in accordance with SEQ ID NO:1 or SEQ ID NO:3.

The detection of a cell that produces LTBP-2 or LTBP-3-encoding DNA segment using a method based upon the sequence of an *ltbp-2* or *ltbp-3* transcript requires an *ltbp-2* or *ltbp-3* probe with a novel DNA sequence as disclosed herein. This imparts an evident utility

to the nucleic acid segments of the present invention, particularly the shorter ones.

5 The presence of a substantially complementary nucleic acid sequence in a sample, or a significantly increased level of such a sequence in comparison to the levels in a normal or "control" sample, will thus be indicative of a sample that contains a cell that harbors an LTBP-2 or LTBP-3-encoding DNA segment. Here, substantially complementary LTBP-2 or LTBP-3-encoding nucleic acid sequences are those that have relatively little sequence divergence and that are capable of hybridizing under relatively stringent conditions, as discussed above.

10 A variety of hybridization techniques and systems are known that can be used in connection with the detection aspects of the invention, including diagnostic assays such as those described in Falkow *et al.*, U.S. Patent 4,358,535. Short coding or non-coding nucleic acid segment probes may also be employed as primers in connection with diagnostic PCR™ technology, as well as for use in any of a number of other PCR™ applications, including PCR™-based cloning and engineering protocols.

15 In general, the "detection" of an LTBP-2 or LTBP-3-encoding DNA segment is accomplished by attaching or incorporating a detectable label into the nucleic acid segment used as a probe and "contacting" a sample with the labeled probe. In such processes, an effective amount of a nucleic acid segment that comprises a detectable label (a probe), is brought into direct juxtaposition with a composition containing target nucleic acids. Hybridized nucleic acid complexes may then be identified by detecting the presence of the label, for example, by detecting a radio, enzymatic, fluorescent, or even chemiluminescent label.

20 Many suitable variations of hybridization technology are available for use in the detection of nucleic acids, as will be known to those of skill in the art. These include, for example, *in situ* hybridization, Southern blotting and Northern blotting. *In situ* hybridization describes the techniques wherein the target nucleic acids contacted with the probe sequences

are those located within one or more cells, such as cells within a clinical sample or even cells grown in tissue culture. As is well known in the art, the cells are prepared for hybridization by fixation, *e.g.*, chemical fixation, and placed in conditions that allow for the hybridization of a detectable probe with nucleic acids located within the fixed cell.

5

Alternatively, target nucleic acids may be separated from a cell or sample prior to contact with a probe. Any of the wide variety of methods for isolating target nucleic acids may be employed, such as cesium chloride gradient centrifugation, chromatography (*e.g.*, ion, affinity, magnetic), phenol extraction and the like. Most often, the isolated nucleic acids will be separated, *e.g.*, by size, using electrophoretic separation, followed by immobilization onto a solid matrix, prior to contact with the labelled probe. These prior separation techniques are frequently employed in the art and are generally encompassed by the terms "Southern blotting" and "Northern blotting". Although the execution of various techniques using labeled probes to detect LTBP-2 or LTBP-3-encoding DNA or RNA sequences in clinical samples are well known to those of skill in the art, a particularly preferred method is described in detail herein, in Examples 1 and 3.

Kits for use in Southern and Northern blotting to identify LTBP-2 or LTBP-3-encoding DNA segments are also contemplated to fall within the scope of the present invention. Such kits will generally comprise, in suitable container means, *ltbp-2* or *ltbp-3*

nucleic acid probes; unrelated probes for use as controls; and optionally, one or more restriction enzymes.

12. Construction of Chimeric Promoter-Reporter Expression Plasmids

Using the 5' upstream flanking sequence of the LTBP-2 and LTBP-3 genes, a series of restriction fragments from available phage genomic inserts may be generated and subcloned into a promoter-reporter expression plasmid. An example of such a vector is pGL3, a luciferase reporter vector (Promega), which has a strong 5' terminator of transcription, a multiple cloning site, the cDNA coding sequence of insect luciferase, a strong translation stop codon, and an intron/polyadenylation signal sequence derived from SV40.

By assembling 9-15 overlapping promoter-reporter expression plasmids that cover an about 5 kb region of interest, the identification of potential *cis*-acting elements is contemplated. Once the initial constructs have been characterized, regions of 5' upstream flanking sequence that show strong promotion (or repression) of gene expression may be studied in careful detail.

The Northern analysis and tissue *in situ* hybridization data presented herein suggest that *ltbp-3* is highly expressed in developing mouse tissues. Additionally, the *ltbp-3* transcript appears to be highly expressed by MC3T3-E1 cells in culture. The structural features of this sequence are consistent with a so-called housekeeping gene, *i.e.*, a TATA-less and CAATT-less promoter sequence that is 70% GC-rich.

To overcome potential problems of low reporter gene expression, the inventors contemplate the use of pEU-CAT, a promoter-less CAT vector especially constructed for the analysis of weak promoters (Harduin-Lepers *et al.*, 1993). Alternatively, modifying the reporter constructs by the addition of an *ltbp-3* enhancer element is also contemplated to be

useful.

13. Evaluating Promoter Function

Promoter function may be evaluated by *in vitro* transfection studies using L cells or NIH3T3 cells, since they have been used successfully for this purpose. An interesting alternative is to use *Drosophila* SL2 cells which: lack the trans-acting factor Sp1; co-transfection of SL2 cells with a plasmid encoding the Sp1 protein such as pP_{ac}Sp1 and a reporter construct containing putative Sp1 binding sites will result in reporter expression if the sites are functional (Courey and Tjian, 1988). L cell transfection is performed using standard protocols (Sambrook *et al.*, 1989). Briefly, subconfluent cells (covering ~20% of a 100 mm plastic tissue culture dish) are washed 2x in DMEM tissue culture medium (GIBCO) and then incubated for 3 hrs. at 37°C in a sterile mixture of DEAE-dextran (0.25 mg/ml), chloroquine (55 mg/ml), and 15 µg plasmid DNA (Courey and Tjian, 1988). Cells are then shocked by incubation with 10% DMSO in sterile PBS for 2 min. at 37°C, washed 2x with DMEM (Sambrook *et al.*, 1989), and incubated in DMEM plus 10% fetal calf serum and antibiotics for 72 hr. at 37°C. Since DEAE-dextran is quite toxic to NIH3T3 cells, these cells may be transfected by electroporation.]

Using the pGL3 reporter/expression vector, luciferase activity may be measured in cell lysates using Luciferase Assay System kit reagents (Promega) according to protocols provided by the manufacturer. To measure background CPM, 20 µl of cell lysate is added to a clean microcentrifuge tube and light activity is measured in a scintillation counter (System 1400 scintillation counter, Wallac Nuclear; all channels open). The same procedure is used to measure background CPM of 100 µl luciferase substrate stock solution. Once the background CPM have been documented, homogenate and substrate are mixed and light emission is measured immediately. Enzyme activity values are routinely obtained in triplicate, normalized to 1 mg of total protein, and expressed as a mean value \pm the standard deviation. Student's *t* test is used to determine statistical significance of differences among

groups (95% confidence level).

In one study, following transfection of COS cells with the pGL3 luciferase expression plasmid, an aliquot of a cell homogenate was assayed for enzyme activity using commercially available kits and according to the manufacturer's recommendations. An equal aliquot of a cell homogenate prepared from untransfected COS served as a negative control. Significant luciferase activity was found only in the homogenate from transfected cells.

14. Biological Functional Equivalents

As mentioned above, modification and changes may be made in the structure of an osteotropic gene and still obtain a functional molecule that encodes a protein or polypeptide with desirable characteristics. The following is a discussion based upon changing the amino acids of a protein to create an equivalent, or even an improved, second-generation molecule. The amino acid changes may be achieved by changing the codons of the DNA sequence, according to the following codon table:

Table 1

Amino Acids			Codons						
Alanine	Ala	A	GCA	GCC	GCG	GCU			
Cysteine	Cys	C	UGC	UGU					
Aspartic acid	Asp	D	GAC	GAU					
Glutamic acid	Glu	E	GAA	GAG					
Phenylalanine	Phe	F	UUC	UUU					
Glycine	Gly	G	GGA	GGC	GGG	GGU			
Histidine	His	H	CAC	CAU					
Isoleucine	Ile	I	AUA	AUC	AUU				
Lysine	Lys	K	AAA	AAG					
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU	
Methionine	Met	M	AUG						
Asparagine	Asn	N	AAC	AAU					
Proline	Pro	P	CCA	CCC	CCG	CCU			
Glutamine	Gln	Q	CAA	CAG					
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU	
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU	
Threonine	Thr	T	ACA	ACC	ACG	ACU			
Valine	Val	V	GUA	GUC	GUG	GUU			
Tryptophan	Trp	W	UGG						
Tyrosine	Tyr	Y	UAC	UAU					

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated by the inventors that various changes may be made in the DNA sequences of osteotropic genes without appreciable loss of their biological utility or activity.

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, incorporate herein by reference). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics (Kyte and Doolittle, 1982), these are: Isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, *i.e.*, still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate ($+3.0 \pm 1$);

glutamate (+3.0 \pm 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0);
threonine (-0.4); proline (-0.5 \pm 1); alanine (-0.5); histidine *-0.5); cysteine (-1.0);
methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3);
phenylalanine (-2.5); tryptophan (-3.4).

5

It is understood that an amino acid can be substituted for another having a similar
hydrophilicity value and still obtain a biologically equivalent, and in particular, an
immunologically equivalent protein. In such changes, the substitution of amino acids whose
hydrophilicity values are within ± 2 is preferred, those which are within ± 1 are particularly
10 preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the
relative similarity of the amino acid side-chain substituents, for example, their
hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which
take various of the foregoing characteristics into consideration are well known to those of
skill in the art and include: arginine and lysine; glutamate and aspartate; serine and
threonine; glutamine and asparagine; and valine, leucine and isoleucine.

* * * * *

The following examples are included to demonstrate preferred embodiments of the
invention. It should be appreciated by those of skill in the art that the techniques disclosed
in the examples which follow represent techniques discovered by the inventors to function
well in the practice of the invention, and thus can be considered to constitute preferred
25 modes for its practice. However, those of skill in the art should, in light of the present
disclosure, appreciate that many changes can be made in the specific embodiments which are

disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE I

ISOLATION OF A NOVEL LATENT TGF- β BINDING PROTEIN-LIKE LTBP-3 GENE

The TGF- β s represent a family of structurally related molecules with diverse effects on mammalian cell shape, growth, and differentiation (Roberts and Sporn, 1990). Initially synthesized as a precursor consisting of an amino-terminal propeptide followed by mature TGF- β , two chains of nascent pro-TGF- β associate in most tissues to form a Mr ~106,000 inactive disulfide-bonded dimer. Homodimers are most common, but heterodimers have also been described (Cheifetz *et al.*, 1987; Ogawa *et al.*, 1992). During biosynthesis the mature TGF- β dimer is cleaved from the propeptide dimer. TGF- β latency results in part from the non-covalent association of propeptide and mature TGF- β dimers (Pircher *et al.*, 1984 and 1986; Wakefield *et al.*, 1987; Millan *et al.*, 1992; see also Miyazono and Heldin, 1989). Consequently, the propeptide dimer is often referred to as the latency associated protein (LAP), and LAP plus the disulfide-bonded TGF- β dimer are also known as the small latent complex. In the extracellular space small latent complexes must be dissociated to activate mature TGF- β . The mechanism of activation of the latent complex is thought to be one of the most important steps governing TGF- β effects (Lyons *et al.*, 1988; Antonelli-Orlidge *et al.*, 1989; Twardzik *et al.*, 1990; Sato *et al.*, 1993).

In certain lines of cultured cells small latent growth factor complexes may contain additional high molecular weight proteins. The best characterized of these high molecular weight proteins is the latent TGF- β binding protein, or LTBP (Miyazono *et al.*, 1988; Kanzaki *et al.*, 1990; Tsuji *et al.*, 1990; Olofsson *et al.*, 1992; Taketazu *et al.*, 1994). LTBP produced by different cell types is heterogeneous in size, perhaps because of alternative splicing or because of tissue-specific proteolytic processing (Miyazono *et al.*, 1988; Wakefield *et al.*, 1988; Kanzaki *et al.*, 1990; Tsuji *et al.*, 1990). Latent TGF- β

complexes that contain LTBP are known as large latent complexes. LTBP has no known covalent linkage to mature TGF- β , but rather it is linked by a disulfide bond to LAP.

Two LTBPs have been isolated to date. The deduced human LTBP-1 amino acid sequence is comprised of a signal peptide, 16 epidermal growth factor-like repeats with the potential to bind calcium (EGF-CB repeats), 2 copies of a unique motif containing 8 cysteine residues, an RGD cell attachment motif, and an 8 amino acid motif identical to the cell binding domain of the laminin B2 chain (Kanzaki *et al.*, 1990). There is evidence that LTBP-1 binds calcium, which, in turn, induces a structural change that protects LTBP from proteolytic attack (Colosetti *et al.*, 1993). LTBP-2 shows 41% sequence identity to LTBP-1, and its structural domains show a similar overall organization (Moren *et al.*, 1994).

While the functions of LTBP-1 and LTBP-2 presently are unknown, several ideas have been put forward in the literature. First, LTBP may regulate the intracellular biosynthesis of latent TGF- β precursors. Cultured erythroleukemia cells efficiently assemble and secrete large latent TGF- β complexes, whereas they slowly secrete small latent TGF- β complexes that contain anomalous disulfide bonds (Miyazono *et al.*, 1991; 1992). Therefore, LTBP may facilitate the normal assembly and secretion of latent TGF- β complexes. Second, LTBP may target latent TGF- β to specific types of connective tissue. Recent evidence suggests that the large latent TGF- β complex is covalently bound to the extracellular matrix via LTBP (Taipale *et al.*, 1994). Based on these observations, LTBP has been referred to as a "matrix receptor", *i.e.* a secreted protein that targets and stores latent growth factors such as TGF- β to the extracellular matrix. Third, LTBP may modulate the activation of latent complexes. This idea is based in part on recent evidence which suggests that mature TGF- β is released from extracellular storage sites by proteases such as plasmin and thrombin and that LTBP may protect small latent complexes from proteolytic attack (Falcone *et al.*, 1993; Benezra *et al.*, 1993; Taipale *et al.*, 1994), *i.e.* protease activity may govern the effect of TGF- β in tissues, but LTBP may modulate this activity. Fourth, LTBP may play an important role in targeting the latent TGF- β complex to the cell surface, allowing latent TGF- β to be efficiently activated (Flaumenhaft *et al.*, 1993).

A. MATERIALS AND METHODS

1. cDNA Cloning

5 Aliquots (typically 40-50,000 PFU) of phage particles from a cDNA library in the
λZAPII® vector made from NIH 3T3 cell mRNA (Stratagene) and fresh overnight
XL1-Blue™ cells (grown in Luria broth supplemented with 0.4% maltose in 10 mM MgSO₄)
were mixed, incubated for 15 min. at 37°C, mixed again with 9 ml of liquid (50°C) top
layer agarose (NZY broth plus 0.75% agarose), and then spread evenly onto freshly poured
10 150 mm NZY-agar plates. Standard methods were used for the preparation of plaque-lifts and
filter hybridization (42°C, in buffer containing 50% formamide, 5X SSPE, 1X Denhardt's,
0.1% SDS, 100 mg/ml salmon sperm DNA, 100 mg/ml heparin). Filters were washed
progressively to high stringency (0.1X SSC/0.1% SDS, 65°C). cDNA probes were
radiolabeled by the nick translation method using commercially available reagents and
15 protocols (Nick Translation Kit, Boehringer Mannheim). Purified phage clones were
converted to pBluescript® plasmid clones, which were sequenced using Sequenase (v2.0) as
described (Chen *et al.*, 1993; Yin *et al.*, 1995). Sequence alignment and identity was
determined using sequence analysis programs from the Genetics Computer Group
(MacVector).

2. Tissue *In Situ* Hybridization

20 To prepare normal sense and antisense probes, a unique 342 bp fragment from the 3'
untranslated region (+3973 to +4314, counting the "A" of the initiator Met codon as +1;
25 see "ish", FIG. 1) was subcloned into the pBSKS+ plasmid (Stratagene, Inc.). Template
DNA was linearized with either *Eco*RI or *Bam*HI, extracted, and precipitated with ethanol.
Sense and antisense transcripts were generated from 1 mg template with T3 and T7
polymerases in the presence of [³⁵S]UTP at >6 mCi/ml (Amersham, >1200 Ci/mmol) and
1.6 U/ml RNasin (Promega), with the remaining *in vitro* transcription reagents provided in a

kit (SureSite, Novagen Inc.). After transcription at 37°C for 1 h, DNA templates were removed by a 15 min. digest at 37°C with 0.5 U/ml RNase-free DNase I, extracted, and precipitated with ethanol. Riboprobes were hydrolyzed to an average final length of 150 bp by incubating in 40 mM NaHCO₃, 60 mM Na₂CO₃, 80 mM DTT for ~40 min. at 60°C. Hydrolysis was terminated by addition of sodium acetate, pH 6.0, and glacial acetic acid to .09 M and 0.56% (v/v), respectively, and the probes were then ethanol precipitated, dissolved in 0.1 M DTT, counted, and stored at -20°C until use. Day 8.5-9.0, day 13.5, and day 16.5 mouse embryo tissue sections (Novagen) and the *in situ* hybridization protocol were exactly as described (Chen *et al.*, 1993; Yin *et al.*, 1995).

3. Northern Analysis

MC3T3-E1 cell poly(A⁺) RNA (2-10 mg aliquots) was electrophoresed on a 1.25% agarose/2.2 M formaldehyde gel and then transferred to a nylon membrane (Hybond-N, Amersham). The RNA was cross-linked to the membrane by exposure to a UV light source (1.2×10^6 mJ/cm², UV Stratalinker 2400, Stratagene) and then pre-hybridized for >15 min. at 65°C in Rapid-Hyb buffer (Amersham, Inc.). A specific cDNA probe consisting solely of untranslated sequence from the 3' end of the transcript was ³²P-labeled by random priming and used for hybridization (2 h at 65°C). Blots were washed progressively to high stringency (0.1X SSC/0.1% SDS, 65°C), and then placed against x-ray film with intensifying screens (XAR, Kodak) at -86°C.

4. Antibody Preparation

LTBP-3 antibodies were raised against a unique peptide sequence found in domain #2 (amino acids 155-167). Peptide #274 (GESVASKHAIYAVC) (SEQ ID NO:5) was synthesized using an ABI model 431A synthesizer employing FastMoc chemistry. The sequence was confirmed using an ABI473 protein sequencer. A cysteine residue was added to the carboxy-terminus to facilitate crosslinking to carrier proteins. For antibody production, the synthetic peptide was coupled to rabbit serum albumin (RSA) using MBS

(*m*-maleimidobenzoic acid-N-hydroxysuccinimide ester) at a substitution of 7.5 mg peptide per mg of RSA. One mg of the peptide-RSA conjugate in 1 ml of Freund's complete adjuvant was injected subcutaneously at 10 different sites along the backs of rabbits. Beginning at 3 weeks after initial immunization, the rabbits were given bi-weekly booster injections of 1 mg peptide-RSA in 100 μ l of Freund's incomplete adjuvant. IgG was prepared by mixing immune serum with caprylic acid (0.7 ml caprylic acid per ml serum), stirring for 30 min., and centrifuging at $5,000 \times g$ for 10 min. The supernatant was decanted and dialyzed against two changes of phosphate buffered saline (PBS) overnight at 4°C. The antibody solution was then affinity purified by passing it over a column containing the immunizing peptide coupled to Affi-gel 10 affinity support. Bound antibodies were eluted with 0.2 M glycine (pH 2.3), immediately dialyzed against PBS, and concentrated to 1 mg/ml. prior to storage at -70°C.

5. Transfection

Transient transfection was performed using standard protocols (Sambrook *et al.*, 1989). Briefly, subconfluent cells (covering ~20% of a 100 mm plastic tissue culture dish) were washed 2x in DMEM tissue culture medium (GIBCO) and then incubated for 3 hrs. at 37°C in a sterile mixture of DEAE-dextran (0.25 mg/ml), chloroquine (55 mg/ml), and 15 mg plasmid DNA (Courey and Tjian, 1988). Cells then were shocked by incubation with 10% DMSO in sterile PBS for 2 min. at 37°C, washed 2x with DMEM (Sambrook *et al.*, 1989), and incubated in DMEM plus 10% fetal calf serum and antibiotics for 72 hr. at 37°C.

6. Immunoprecipitation

For immunoprecipitation, 1 ml of antibody (1:400 final concentration, in PBS-TDS buffer: 0.38 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 1% Triton X-100, 0.5% Deoxycholic acid, and 0.1% SDS) was added to 1 ml of radiolabeled medium proteins. The mixture was incubated with shaking at 4°C for 1 hr., protein A-sepharose CL-4B beads were added (200 μ l, 10% suspension), and this mixture was incubated with

shaking for one additional hour at 4°C. Immunoprecipitated proteins were pelleted by brief centrifugation, the pellet was washed 6x with PBS-TBS buffer, 2x protein loading dye was added, and the samples were boiled for 5 min. and then fractionated on 4-18% gradient SDS-PAGE (Bonadio *et al.*, 1985). Cold molecular weight markers (200 kDa-14.3 kDa, Rainbow mix, Amersham) were used to estimate molecular weight. The gel was dried and exposed to film for the indicated time at room temperature.

7. Western Analysis

Fractionated proteins within SDS-polyacrylamide gels were transferred to a nitrocellulose filter for 2 hours using Tris-glycine-methanol buffer, pH 8.3 at 0.5 mA/cm². The filter was blocked, incubated with nonfat milk plus antibody (1:1000 dilution) for 2 hr, and washed. Antibody staining was visualized using the ECL Western blotting reagent (Amersham) according to the manufacturer's protocols.

B. RESULTS

In this study, the inventors isolated and characterized a novel murine fibrillin-like cDNA encoding LTBP-3. To clone the murine LTBP-3 gene, cDNA from a 3T3 cell cDNA library was amplified using human fibrillin-1 PCR™ primers under low stringency conditions (*i.e.*, annealing at 37°C initially for 10 cycles, followed by annealing at 60°C for 30 cycles). The results indicated that a murine DNA fragment of unexpectedly low homology (~50%) to human fibrillin-1 was obtained. Molecular cloning of the authentic murine fibrillin-1 transcript was also performed, confirming the human and murine fibrillin-1 coding sequences share >95% sequence identity. The murine fibrillin-1 and PCR™ sequences were different, which suggested that the PCR™ product may have been derived from a related, fibrillin-like cDNA. The 3T3 cell cDNA library was screened at high stringency using the murine PCR™ product as the probe in order to test this hypothesis. A cDNA walking strategy eventually yielded seven overlapping cDNA clones (FIG. 1). It provides a unique mRNA of 4,314 nucleotides, with an open reading frame of 3,753 nucleotides (SEQ ID NO:3) (FIG. 9). The

deduced molecule is a unique polypeptide of 1,251 amino acids (SEQ ID NO:4) (FIG. 10). Excluding the signal peptide (21 amino acids), the novel fibrillin-like molecule consists of five structurally distinct regions (Region 1- Region 5), and although similar to murine fibrillin-1 (FIG. 2A), its domain structure is unique as is evidenced by the schematic representation of LTBP-3 shown in FIG. 2B.

Domain #1 is a 28 amino acid segment with a net basic charge (est pI, 12.36) that may allow for binding acidic molecules in the extracellular matrix (*e.g.*, acidic proteoglycans). Sequences rich in basic amino acids may also function as endoproteolytic processing signals (Barr, 1991; Steiner *et al.*, 1992), which suggests that the NH₂-terminus may be proteolytically processed. Domain #2 extends for 390 amino acids, consisting of an EGF-like repeat, a 135 amino acid segment that was proline-rich (20.7%) and glycine-rich (11.8%) but not cysteine-rich, a Fibmotif (Pereira *et al.*, 1993), an EGF-CB repeat, and a TGF-bp repeat. Domain #3 is a 113 amino acid segment characterized by its high proline content (21%). Domain #4 extends for 678 amino acids and consists of 14 consecutive cysteine-rich repeats. Based on structural homologies, 12/14 repeats were epidermal growth factor-calcium binding (EGF-CB) motifs (Handford *et al.*, 1991), whereas 2/14 were transforming growth factor- β -binding protein (TGF-bp) motifs (Kanzaki *et al.*, 1990). Finally, domain #5 is a 22 amino acid segment at the carboxy-terminus. The conceptual amino acid sequence encoded by the open reading frame consisted of 1,251 amino acids (FIG. 2B) with an estimated pI of 5.92, a predicted molecular mass of 134,710 Da, and five potential N-linked glycosylation sites. No RGD sequence was present.

Northern blot analysis of murine embryo RNA using a 3' untranslated region probe identified a transcript band of ~4.6 kb. In this regard, 4,310 nt have been isolated by cDNA cloning, including a 3' untranslated region of 401 nt and a 5' upstream sequence of 156 nt. The apparent discrepancy between the Northern analysis result and the cDNA sequence analysis suggested that the 5' upstream sequence may include ~300 nt of additional upstream sequence. This estimate was consistent with preliminary primer extension mapping

studies indicating that the 5' upstream sequence is 400-500 nt in length.

5 A total of 19 cysteine-rich repeats were found in domains #2 and #4 of the murine LTBP-like (LTBP-3) polypeptide. Thirteen were EGF-like and 11/13 contained the calcium binding consensus sequence. This consensus was derived from an analysis of 154 EGF-CB repeats in 23 different proteins and from structural analyses of the EGF-CB repeat, both bound and unbound to calcium ion (Selander-Sunnehaugen *et al.*, 1992). Variations on the consensus have been noted previously and one of these, D-L-N/D-E-C₁, was identified in the third EGF-like repeat of domain #4. In addition, a potential calcium binding sequence which has not previously been reported (E-T-N/D-E-C₁) was identified in the first EGF-like repeat of domain #4. Ten of thirteen EGF-CB repeats also contained a second consensus sequence which represents a recognition sequence for an Asp/Asn hydroxylase that co- and post-translationally modifies D/N residues (Stenflo *et al.*, 1987; Gronke *et al.*, 1989).

10 Although about one-half the size, the deduced polypeptide was organized like fibrillin-1 in that it consisted of a signal peptide followed by 5 structurally distinct domains, *i.e.*, two domains with numerous EGF-like, EGF-CB and Fib repeats and a third with a proline-rich sequence (Pereira *et al.*, 1993). However, comparison of each of these domains using the GAP and BESTFIT programs (Genetics Computer Group) has revealed a low level of amino acid homology of only 27% over the five structural domains shared by the deduced murine polypeptide and human fibrillin-2. These values are low for a putative fibrillin family member because fibrillin-1 and fibrillin-2 share ~50% identity (Zhang *et al.*, 1994).

25 A search of available databases revealed that the deduced murine polypeptide was most similar to the human and rat latent TGF- β binding proteins (Kanzaki *et al.*, 1990; Tsuji *et al.*, 1990). In this regard LTBP-3 was found to be similar to fibrillin in that it could also be divided into five structurally distinct domains (FIG. 2A, FIG. 2B, and FIG. 2C). These include a relatively short domain downstream of the signal peptide with a net basic charge (amino acids 21-33, est. pI, 11.14); a domain consisting of EGF-like, EGF-CB, TGF-bp, and

Fib motifs plus a proline-rich and glycine-rich sequence (amino acids 34-407); a proline-rich domain (amino acids 408-545); a large, domain consisting of EGF-CB, TGF-bp, and TGF-bp-like repeat motifs (amino acids 546-1379); and a relatively short domain at the carboxy terminus (amino acids 1380-1394). Amino acid sequence comparison of the deduced murine and human polypeptides shows 60% identity for domain #1, 52% identity for domain #2, 30% identity for domain #3, 43% identity for domain #4, and 7% identity for domain #5. The average identity over the five domains shared by the murine polypeptide and human LTBP was 38.4%. Significantly, cysteine residues in both polypeptide sequences were highly conserved.

The fibrillins are exclusively expressed by connective cells in developing tissues (Zhang *et al.*, 1994), whereas LTBP should be expressed along with TGF- β by both epithelial and connective cells (Tsuji *et al.*, 1990). The structural homology data therefore predict that the murine LTBP-3 gene shown in FIG. 2B should be expressed by both epithelial and connective tissue cells. Tissue *in situ* hybridization was used to test this hypothesis.

An overview of the expression pattern as determined by tissue *in situ* hybridization is presented in FIG. 3A, FIG. 3B, FIG. 3C, FIG. 3D, FIG. 3E and FIG. 3F.

Approximate mid-sagittal sections of normal murine embryos at days 8.5-9.0, 13.5 and 16.5 *p.c.* of development were hybridized with a ^{35}S -labeled single stranded normal sense riboprobe from the same cDNA construct was used. At day 8.5-9.0 of development, intense gene expression was observed in the mesometrial and anti-mesometrial uterine tissues, ectoplacental cone, placenta, placental membranes. The transcript appeared to be widely expressed in murine embryo mesenchymal/connective tissue compartments, including the facial mesenchyme, at days 8.5-9.0, 13.5 and 16.5 of development. Particularly intense expression of the transcript was noted in the liver.

Microscopy of day 8.5-9.0 embryos confirmed the widespread expression of the murine gene by mesenchymal cells. Significant expression of the transcript by cells of the developing central nervous system, somites and cardiovascular tissue (myocardium plus endocardium) was also observed.

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Microscopy of day 13.5 and day 16.5 embryos demonstrated expression of the murine gene by skeletal muscle cells and by cells involved in intramembranous and endochondral bone formation. The transcript was expressed by osteoblasts and by periosteal cells of the calvarium, mandible and maxilla. The transcript was also identified in both cartilage and bone of the lower extremity. A positive signal was detected in perichondrial cells and chondrocytes (proliferating > mature > hypertrophic) of articular cartilage, the presumptive growth plate, and the cartilage model within the central canal. The positive signal was also expressed by blood vessel endothelial cells within the mid-diaphysis, and the surrounding muscle cells.

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Respiratory epithelial cells lining developing small airways and connective tissue cells in the pulmonary interstitium expressed the murine transcript, as did myocardial cells (atria and ventricles) and endocardial cushion tissue. Cells within the walls of large arteries also expressed the transcript. Expression of the murine gene was identified in several organs of the alimentary system, including the tongue, esophagus, stomach, small and large intestine, pancreas and liver. Mucosal epithelial cells lining the upper and lower digestive tract plus the smooth muscle and connective tissue cells found in the submucosa expressed the transcript, as did acinar cells of the exocrine pancreas. Despite the high level of transcript expression in the liver, these results suggest both cell populations express the LTBP-3 transcript.

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In the kidney, expression above the basal level was observed in cells of developing nephrons, the ureteric bud, kidney blastema and the kidney interstitium. In the skin, epidermal and adnexal keratinocytes, dermal connective tissue cells, and brown fat cells within the dorsal subcutis expressed the murine transcript. In the central and peripheral

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nervous systems, ganglion cells within the cerebrum, brainstem, spinal cord, and peripheral nerves expressed the murine transcript. The transcript was also intensely expressed by cells of the developing murine retina.

Thus, the murine gene is widely expressed by both epithelial and connective tissue cells, a pattern that would be expected for a latent TGF- β binding protein. Three final observations argue that the LTBP-like (LTBP-3) sequence presented herein is not simply the murine homologue of human LTBP. First, domain #4 of the murine LTBP-like (LTBP-3) sequence has a smaller number of EGF-like repeat motifs than human and rat LTBP (8 versus 11). Second, portions of the human and rat LTBP-like coding sequence were characterized and found to share ~90% identity with human and rat LTBP but only 65% identity with the murine LTBP-like gene. Third, the human LTBP and LTBP-like genes are localized to separate chromosomes. Human LTBP was assigned to human chromosome 2 based on the analysis of human \times rodent somatic cell hybrid lines (Stenman *et al.*, 1994). The present invention represents the first mapping of an LTBP gene in the murine. The human LTBP-like genes was recently localized to chromosome 11 band q12, while the murine gene was mapped to murine chromosome 19, band B (a region of conserved synteny), using several independent approaches, including fluorescent *in situ* hybridization.

The first indication of alternative splicing came from molecular cloning studies in the murine, in which independent cDNA clones were isolated with a deletion of 51 bp from the coding sequence. PCR[™]/Southern blot analysis provided additional evidence that the homologous 51 bp sequence was alternatively spliced in normal murine embryo tissues.

Northern blot analysis also demonstrated that the novel fibrillin gene was also expressed in rat callus three weeks after osteotomy, after mineralization has begun. Gene expression in normal adult rat bone tissue was insignificant, which suggests that microfibrils are an important part of the bone fracture healing response. The novel fibrillin-like gene was expressed in callus as a pair of alternatively spliced transcripts. This result has been independently reproduced on three occasions. Molecular cloning of the novel fibrillin gene

in both murine and rat has identified potential splice junction sites for the alternative splicing event.

MC3T3-E1 murine pre-osteoblasts were used to demonstrate that the murine gene product was capable of binding TGF- β . MC3T3-E1 cells were utilized because they synthesize and secrete TGF- β , which may act as an autocrine regulator of osteoblast proliferation (Amarnani *et al.*, 1993; Van Vlasselaer *et al.*, 1994; Lopez-Casillas *et al.*, 1994).

To determine whether or not MC3T3-E1 cells co-expressed the murine gene product of TGF- β , cells were plated on 100-mm dishes under differentiating conditions (Quarles *et al.*, 1992) and the medium was replaced twice weekly. Parallel dishes were plated and assayed for cell number and alkaline phosphatase activity, which confirmed that osteoblast differentiation was indeed taking place. Equal aliquots of total cellular RNA was prepared from these MC3T3-E1 cells after 5, 14 and 28 days in culture for Northern blot analysis. As shown in FIG. 4, expression of the new murine gene peaked on day 14 of culture. Since MC3T3-E1 cells also show a peak in alkaline phosphatase activity on day 14 of culture (Quarles *et al.*, 1992), the results suggest for the first time that LTBP-3 gene expression is an early marker of osteoblast differentiation.

C. DISCUSSION

This study reports the molecular cloning of a novel LTBP-3 gene that contains numerous EGF-like repeats. Northern analysis indicates that the gene encodes a single transcript of ~4.6 kb in murine embryo tissues. The deduced amino acid sequence of the murine gene product appears to be a secreted polypeptide of 1,251 amino acids. Although it is similar to fibrillin, the overall structural organization and expression pattern of this gene product most resembles LTBP, a latent TGF- β binding protein that was originally isolated and characterized by Heldin and co-workers (Kanzaki *et al.*, 1990). Several observations strongly suggest that LTBP and the murine LTBP-like gene product are therefore derived

from related but distinct genetic loci. First, LTBP and the LTBP-like coding sequence share ~40% identity and differences exist in the number of EGF-CB repeats in the deduced polypeptide sequence of the two molecules. Second, a portion of the murine LTBP gene has been cloned and shown to share ~90% identity with human and rat LTBP. Conversely, portions of the human and rat LTBP-like genes have been cloned and shown to share ~90% identity with the murine LTBP-like gene. Third, LTBP and the LTBP-like gene reside on different human chromosomes (Stenman *et al.*, 1994). Taken together, these data suggest that a family of at least two LTBP genes exists.

Similarities in the structural organization of LTBP-1 and the fibrillin-1 and fibrillin-2 polypeptides have been noted previously (Pereira *et al.*, 1993; Zhang *et al.*, 1994; Taipale *et al.*, 1994). For example, LTBP-1 and the fibrillins are all secreted extracellular matrix constituents. Moreover, each polypeptide can be organized into five domains, two of which consists predominantly of EGF-CB and TGF- β p repeat motifs. LTBP-1 and fibrillin-1 also share a domain that is proline-rich, and LTBP possesses an 8-cysteine repeat previously referred to as the "Fib motif" because it was assumed to be unique to fibrillin (Pereira *et al.*, 1993). These similarities likely explain the initial isolation and cloning of the LTBP-3 PCRTM product, especially since the human oligonucleotide primers used to initially amplify murine cDNA were designed to direct the synthesis of an EGF-CB repeat in domain #4.

Another point of distinction between LTBP-3 and fibrillin concerns the spacing of conserved cysteines C4 and C5 in EGF-like repeats. Fibrillin-1 and fibrillin-2 each contain >50 such repeats, and in every one the spacing is C₄-X-C₅. While this pattern is repeated in a majority of the EGF-like repeats in LTBP-1 and LTBP-2, both genes also contain repeats with the spacing C₄-X-X-C₅. Although the significance of this observation is unclear, variation in the number of amino acids between C₄ and C₅ would not be expected to alter the function of the EGF-like repeat. Mature EGF is a 48 amino acid secreted polypeptide consisting of two subdomains that have few interdomain contacts (Engel, 1989; Davis, 1990). The larger NH₂-terminal subdomain consists of residues 1-32 and is stabilized by a pair of disulfide bonds (C₁-C₃ and C₂-C₄), whereas the smaller COOH-terminal subdomain (amino

acids 33-48) is stabilized by a single disulfide bond (C₅-C₆). The COOH-terminal subdomain has a highly conserved conformation that only is possible if certain residues and the distances between them are well conserved, while conformation-sequence requirements for the NH₂-terminal subdomain are relatively relaxed. Variation in C₄-C₅ spacing would not be expected to alter conformation because these residues do not normally form a disulfide bond and the spacing variation occurs at the interface of subdomains that would not be predicted to interact. The cloning of additional genes will decide whether variation in C₄-C₅ spacing is a reliable discriminator between members of the LTBP and fibrillin gene families.

The LTBP-3 gene is expressed more widely during development than fibrillin-1 or fibrillin-2. Studies in developing murine tissues have shown that the *Fbn-1* gene is expressed by mesenchymal cells of developing connective tissue, whereas the murine LTBP-like gene is intensely expressed by epithelial, parenchymal and stromal cells. Earlier reports have suggested that TGF- β plays a role in differentiation and morphogenesis during murine development (Lyons and Moses, 1990), when TGF- β is produced by epithelial, parenchymal and stromal cells. Tsuji *et al.*, (1990) and others have suggested that the expression of TGF- β binding proteins should mirror that of TGF- β itself; the expression pattern of the LTBP-3 gene over the course of murine development is consistent with this expectation. However, the LTBP-3 gene may not be completely co-regulated with TGF- β . TGF- β gene and protein expression during murine development has been surveyed extensively (Heine *et al.*, 1987; Lehnert and Akhurst, 1988; Pelton *et al.*, 1989; Pelton *et al.*, 1990a, b; Millan *et al.*, 1991); these studies have not identified expression by skeletal muscle cells, chondrocytes, hepatocytes, ganglion cells, mucosal cells lining the gut, and epithelial cells of developing nephrons. It is conceivable that the LTBP-3 molecule has an additional function in certain connective tissues besides targeting TGF- β .

The binding properties of the LTBP-3 gene product are under investigation. Formally, the LTBP-3 polypeptide may bind a specific TGF- β isoform, another member of the TGF- β superfamily (*e.g.*, a bone morphogenetic protein, inhibin, activin, or Mullerian

inhibiting factor), or a growth factor unrelated to TGF- β . Anti-peptide antibodies to the murine LTBP-3 polypeptide have been generated and osteoblast cell lines that express the molecule at relatively high levels have been identified. Studies with these reagents suggest that LTBP-3 assembles intracellularly into large latent complexes with a growth factor that is being characterized by immunological methods.

The presence of dibasic amino acids in the LTBP-3 sequence suggests that it may undergo cell- and tissue-specific proteolysis. TGF- β regulates extracellular matrix production by suppressing matrix degradation (through a decrease in the expression of proteases such as collagenase, plasminogen activator, and stromelysin plus an increase in the expression of proteinase inhibitors such as plasminogen activator inhibitor-1 and tissue inhibitor of metalloproteinase-1) and by stimulating matrix macromolecule synthesis (for recent review, see Lyons and Moses, 1990; Massague, 1990; Laiho and Keski-Oja, 1992; Miyazono *et al.*, 1992). Conversely, production of extracellular matrix has been shown to down regulate TGF- β gene expression (Streuli *et al.*, 1993). TGF- β may therefore regulate extracellular matrix production through a sophisticated feedback loop that influences the expression of a relatively large number of genes. LTBP-1 and LTBP-2 may contribute to this regulation by facilitating the assembly and secretion of large latent growth factor complexes and then targeting the complex to specific connective tissues (Taipale *et al.*, 1994).

If LTBP-3 is like LTBP-1, it has the potential to function as a secreted, extracellular structural protein. As demonstrated here, domain #1 of LTBP-3 appears to be a unique sequence that likely has a globular conformation. Domain #1 also is highly basic and may facilitate LTBP-3 binding to acidic molecules (*e.g.*, acidic proteoglycans) within the extracellular space. Sequences rich in basic amino acids have also been shown to function as endoproteolytic processing signals for several peptide hormones (Barr, 1991; Steiner *et al.*, 1992). It is possible, therefore, that the NH₂-terminus of LTBP-3 is proteolytically processed in a tissue-specific manner.

Domains #2 and #4 consist of consecutive cysteine-rich repeats, the majority of which are of the EGF-CB type. Besides binding calcium (Corson *et al.*, 1993), these repeats may provide LTBP-3 with regions conformation capable of interacting with other matrix macromolecules (Engel, 1989). Domain #3 is proline rich and may be capable of bending (or functioning like a hinge) in three-dimensional space (MacArthur and Thornton, 1991). (In this regard, domain #2 is of interest because it has a similar stretch of 135 amino acids that is both proline- and glycine-rich. Since glycine-rich sequences are also thought to be capable of bending or functioning like a hinge in three-dimensional space, this amino acid sequence may interrupt the extended conformation of domain #2, thereby providing it with a certain degree of flexibility in three-dimensional space.)

Domain #5 also appears to be a unique sequence having a globular conformation. The absence of a known cell attachment motif may indicate that, in contrast to LTBP-1, the LTBP-3 molecule may have a more limited role in the extracellular matrix (*i.e.*, that of a structural protein) in addition to its ability to target latent TGF- β complexes to specific connective tissues.

MC3T3-E1 pre-osteoblasts co-express LTBP-3 and TGF- β 1 and these proteins form a complex in the culture medium. These results are particularly interesting because bone represents one of the largest known repositories of latent TGF- β (200 μ g/kg bone; Seyedin *et al.*, 1986 and 1987), and because this growth factor plays a critical role in the determination of bone structure and function. For example, TGF- β is thought to (i) provide a powerful stimulus to bone formation in developing tissues, (ii) function as a possible "coupling factor" during bone remodeling (a process that coordinates bone resorption and formation), and (iii) exert a powerful bone inductive stimulus following fracture. Activation of the latent complex may be an important step governing TGF- β effects, and LTBP may modulate the activation process (*e.g.*, it may "protect" small latent complexes from proteolytic attack).

Expression of large latent TGF- β complexes bearing LTBP may be physiologically

relevant to, *i.e.*, may be part of the mechanism of, the pre-osteoblast → osteoblast differentiation cascade. This is based on the evidence that MC3T3-E1 cells express large latent TGF- β 1 complexes bearing LTBP-2 precisely at the time of transition from the pre-osteoblast to osteoblast phenotype (~day 14 in culture, or, at the onset of alkaline phosphatase expression; see Quarles *et al.*, 1992). The organ culture model, for example, likely is comprised of differentiated osteoblasts but few bone progenitors, making it a difficult model at best in which to study the differentiation cascade (Dallas *et al.*, 1984). It is also well known that MG63, ROS17/2.8 and UMR 106 cells are rapidly dividing and they express the osteoblast phenotype. Thus, these osteoblast-like cell lines do not show the uncoupling of cell proliferation and cell differentiation that characterizes the normal (physiologically relevant) pre-osteoblast → osteoblast transition (Gerstenfeld *et al.*, 1984; Stein and Lian, 1993). Therefore, the production of small versus large latent TGF- β complexes may be associated with specific stages in the maturation of bone cells.

LTBP-3 may bind calcium, since EGF-CB repeats have been shown to mediate high affinity calcium binding in LTBP-1 and other proteins (Colosetti *et al.*, 1993). Calcium binding, in turn, may contribute to molecular conformation and the regulation of its interactions with other molecules. The presence of dibasic amino acids suggests that LTBP-3 may also undergo cell- and tissue-specific proteolysis. TGF- β regulates extracellular matrix production by suppressing matrix degradation (through a decrease in the expression of proteases such as collagenase, plasminogen activator, and stromelysin plus an increase in the expression of proteinase inhibitors such as plasminogen activator inhibitor-1 and tissue inhibitor of metalloproteinase-1) and by stimulating matrix macromolecule synthesis (for recent reviews, see Lyons and Moses, 1990; Massague, 1990; Laiho and Keski-Oja, 1992; and Miyazono *et al.*, 1993). Conversely, production of extracellular matrix has been shown to down regulate TGF- β gene expression (Streuli *et al.*, 1993). TGF- β may therefore regulate extracellular matrix production through a sophisticated feedback loop that influences the expression of a relatively large number of genes. LTBP-1, LTBP-2, and LTBP-3 may contribute to this regulation by facilitating the assembly and secretion of large latent growth

factor complexes and then targeting the complex to specific connective tissues (Taipale *et al.*, 1994).

EXAMPLE II

PREPARATION OF LTBP-3 ANTIBODIES

An affinity-purified antibody (#274) capable of immunoprecipitating was prepared against the murine LTBP-3 gene product. Full-length murine cDNAs were assembled into the pcDNA3 mammalian expression vector (Invitrogen) and expressed following transient transfection of 293T cells. Nascent polypeptides, radiolabeled by addition of ^{35}S Cys to the medium of transfected cells, were immunoprecipitated using affinity-purified antibody #274. As shown in FIG. 5, the new murine polypeptide was estimated to be 180-190 kDa. To ensure the specificity of #274 binding, preincubation with 10 μg of synthetic peptide was shown to block immunoprecipitation of the 180-190 kDa band.

Finally, MC3T3-E1 cells were cultured for 7 days under differentiating conditions and double-labeled with 30 $\mu\text{Ci/ml}$ ^{35}S cysteine and ^{35}S methionine in deficient media. Radiolabeled media was dialyzed into cold PBS with protease inhibitors. Aliquots of the dialyzed medium sample (10^6 incorporated CPM) were analyzed by a combined immunoprecipitation/Western analysis protocol. The murine polypeptide was clearly and reproducibly secreted by MC3T3-E1 cells, migrating under reducing conditions as a single band of 180-190 kDa (FIG. 6). Consistent with the results of previous studies (*e.g.*, Miyazono *et al.*, 1988; Dallas *et al.*, 1994; Moren *et al.*, 1994), bands of 70 and 50 kDa corresponding to the TGF- β 1 precursor were co-immunoprecipitated with the 180 kDa LTBP-3 protein. Weak bands of 40 and 12 kDa were also identified in experiments in which only immunoprecipitation was performed. The latter were not included in FIG. 6 because they migrated within that portion of the gel included in the Western analysis. Protein bands of 70-12.5 kDa are not variant forms of LTBP-3; FIG. 5 demonstrates that LTBP-3 migrates as a single band of 180-190 kDa following transient transfection of 293T cells, which fail to make TGF- β 1. By immunoprecipitation, a unique band consistent with monomeric mature

TGF- β 1 was found in the LTBP-2 immunoprecipitate. Antibody #274 is incapable of binding TGF- β 1 as determined by radioimmunoassay using commercially available reagents (R&D Systems) and the manufacturer's suggested protocols. These results have been reproduced in 6 independent experiments which utilized 3 separate lots of MC3T3-E1 medium. Thus the new murine LTBP-3 polypeptide binds TGF- β *in vitro*.

In co-transfection studies of 293T Cells using pLTBP-3fl and pTGF- β 1, immunoprecipitation of LTBP-3 and TGF- β 1 was demonstrated by 293T cells following transient transfection and radiolabeling. Aliquots ($\sim 10^6$ incorporated CPM) of radiolabeled media were immunoprecipitated and separated using 4%-18% gradient SDS-PAGE and either reducing or nonreducing conditions as described (Yin et al., 1995) (FIG. 13).

EXAMPLE III

ISOLATION OF A GENE ENCODING MURINE LTBP-2

In addition to determining the DNA and corresponding polypeptide sequence of the murine LTBP-3 gene, the murine LTBP-2 gene was also cloned and sequenced.

1. Cloning and DNA sequencing of LTBP-2

To identify new murine LTBP family members, independently designed degenerate oligonucleotide primers were synthesized based on a structural homology shared by human LTBP-1 and mouse LTBP-3 coding sequences: forward primer, 5'-AAACGTCACACGTGAIACGTGAACGTTGCTTGCTGG-3' (SEQ ID NO:12); reverse primer, 5'-TTACGTCCACGTACACGTCTAGCAAGCAAGCA-3' (SEQ ID NO:13), and then used PCR[™] to amplify single-stranded mouse embryo cDNA prepared from normal CD-1 mouse embryo mRNA. A band of approximately 400 basepairs (bp) was isolated and purified by agarose gel electrophoresis, the DNA was ligated into the TA cloning vector (InVitrogen), and the ligation mixture was used to transform competent bacteria. Plasmid DNA (from 28 colony forming units) was prepared and evaluated by DNA sequence analysis.

As determined by sequence identity comparison, 16/28 plasmid DNAs coded for mouse LTBP-1, 11/28 coded for mouse LTBP-3, and 1/28 coded for an apparently unique sequence. The insert DNA from the unique plasmid was then used as a probe to screen a cDNA library prepared from 3T3 cells (Stratagene, Inc.). A walking strategy eventually yielded the overlapping cDNA clones shown in FIG. 11. Analysis of these clones identified an open reading frame of 5,430 base pairs. Comparison of sequence identity using the GAP and BESTFIT programs (Genetics Computer Group) revealed 79.7% identity between the mouse open reading frame and human LTBP-2 (Centrella *et al.*, 1991), but \leq 47.1% identity between the mouse open reading frame and human LTBP-1 and mouse LTBP-3. The sequence comparison data agreed with chromosomal localization data, which collectively established that the sequence was the mouse homolog of human LTBP-2.

The level of amino acid sequence identity (approximately 40%) among the LTBP-1, -2, and -3 polypeptides is in the range observed for other protein isoforms that contain multiple EGF-like repeats, like the fibrillins (Yin *et al.*, 1995) and the diverse laminin chains (Engel, 1989).

An LTBP-2 methionine codon in a favorable context for translation initiation was provisionally designated the translation start site (see Kozak, 1991). The deduced initiator methionine was followed by a signal sequence of approximately 35 amino acids. Consistent with the structure and length of the human LTBP-2 signal peptide (Centrella *et al.*, 1991), the 15 residues immediately downstream of the mouse LTBP-2 initiator methionine were largely hydrophilic in nature, whereas amino acids 16-35 represented a typical hydrophobic signal peptide sequence. The small neutral amino acid residues Ser (-3) and Ala (-1) and the large polar residue Gln (+1) appeared to define the signal peptide cleavage site (von Heijne, 1983).

In contrast to LTBP-1 and -3, which appear to be organized into 5 structurally distinct domains downstream of the signal peptide (Yin *et al.*, 1995), the deduced mouse LTBP-2 polypeptide consists of ten alternating structural domains that are composed of either

proline- and glycine-rich sequences or cysteine-rich repeat motifs. Thus, domain 1 (amino acids 36-160) was composed of 19.4% glycine and proline residues. Domain 2 (amino acids 161-213) consisted of 2 EGF-like repeats. Domain 3 (amino acids 214-344) was composed of 22.3% glycine and proline residues. Domain 4 (amino acids 345-413) consisted of 2 cysteine-rich repeats. Domain 5 (amino acids 414-536) was composed of 19.5% glycine and proline residues. Domain 6 (amino acids 537-708) consisted of 3 cysteine-rich repeats; based on structural homologies, the first repeat was a Fib motif (Pereira *et al.*, 1993; a copy of this reference has been included in the Appendix), the second was an epidermal growth factor-calcium binding (EGF-CB) motif (Handford *et al.*, 1990), and the third was a transforming growth factor- β 1-binding protein (TGF-bp) motif (Kanzaki *et al.*, 1990). Domain 7 (amino acids 709-831) was composed of 20.3% proline and glycine residues. Domain 8 (amino acids 832-1626) consisted of 15 EGF-like repeats and 2 TGF-bp repeats. Domain 9 (amino acids 1627-1721) was composed of 29.5% glycine and proline residues. Domain 10 (amino acids 1722-1810) consisted of 2 EGF-like repeat motifs.

The conceptual mouse LTBP-2 amino acid sequence consists of 1,810 amino acids, with an estimated *pI* of 5.02, a predicted molecular mass of 197,917 Da., and eight potential *N*-linked glycosylation sites. Similar to the mouse LTBP-3 polypeptide, RGD and laminin B2 chain cell adhesion sequences were not identified. Altogether, 26 cysteine-rich repeats were found in the mouse LTBP-2 polypeptide. As described above, 20/26 were characterized by the presence of 6 cysteine residues and therefore were EGF-like. 12/20 showed the general consensus D/N-I/V-D/N-E/D-C₁, derived from an analysis of 154 EGF-like repeats in 23 different proteins and from structural analysis of the coagulation factor X EGF-CB repeat, both bound and unbound to calcium ion (Selander-Sunnerhagen *et al.*, 1992). Variations on the consensus have been noted previously (for examples see Yin *et al.*, 1995; Yin *et al.*, 1995), and two of these, were identified in mouse LTBP-2. Two potential variants which have not previously been reported were also identified (D-A-D-E-C₁ and D-H-N-E-C₁), giving a total of 16 putative EGF-CB repeats. All 16 repeats also contained a proposed recognition sequence (C₃-X-D/N-X-X-X-X-Y/F-X-C₄) for an Asp/Asn hydroxylase

that co- and posttranslationally modifies D/N residues (Stenflo *et al.*, 1987; Gronke *et al.*, 1989). Previous NMR studies of the isolated first EGF-like domain of human factor IX indicate that 3 residues derived from the general consensus and from the recognition sequence are direct ligands for calcium (Handford *et al.*, 1990; 1995; 1991). This has led to a proposed calcium-binding consensus D/N, D/N, D*/N* (where * denotes a β -hydroxylated residue). A fourth residue in the consensus as originally proposed, F/Y, is now known not to be a direct ligand for calcium (Hughes *et al.*, 1993). The three amino acids that are direct ligands for calcium in factor IX are conserved in each of the 16 putative EGF-CB repeats identified in mouse LTBP-2.

Four of five of the putative domains rich in proline and glycine were also rich in basic amino acid residues (domain 1, 15.5%; domain 3, 13.1%; domain 5, 11%; domain 7, 8.1%; and domain 9, 3.2%). Co-existence of proline and basic amino acids suggests the possibility that LTBP-2 undergoes 'proline-directed' endoproteolytic processing (Devi, 1991). Indeed, both monobasic and dibasic cleavage motifs — *e.g.*, R-R and R-X-X-R, respectively (Barr, 1991)—were identified in all five postulated proline- and glycine-rich structural domains. In several instances, monobasic cleavage motifs occurred near or within potential dibasic cleavage motifs (*e.g.*, Arg 108, Arg 286, Arg 429, and Lys 727). One potential monobasic cleavage motif was identified in each of the 5 proline- and glycine-rich domains. Dibasic cleavage motifs in general were more prevalent near the deduced LTBP-2 amino terminus. Endoproteolytic cleavage of LTBP is of potential interest because it may help explain the smaller than expected size of platelet LTBP-1.

The complete cDNA nucleotide sequence for murine LTBP-2 is shown in (SEQ ID NO:1) (FIG. 7). The deduced amino acid sequence is shown in (SEQ ID NO:2) (FIG. 8).

2. Mouse *ltbp-2* Gene Expression in Developing Perichondrium

The inventors have demonstrated that *ltbp-3* is widely and intensely expressed in both

developing maternal tissues (*e.g.*, uterine decidua) and mouse embryo tissues (*e.g.*, mesenchyme, connective tissue, epithelia, and parenchyma). Tissue *in situ* hybridization was used to compare and contrast the developmental expression of *ltbp-2* and *ltbp-3*, FIG. 12A.. FIG. 12B, and FIG 12C present an overview of *ltbp-2* expression in a mid-sagittal section of a mouse embryo at day 16.5 *p.c.* of development, when expression is strongest. The section was hybridized with a ³⁵S-labeled single stranded antisense riboprobe synthesized from a 580 base pair cDNA coding for the mouse LTBP-2 3' untranslated region. The probe showed <30% sequence identity with the 3' untranslated sequences of human *ltbp-1* and *ltbp-2*, which is too low to give spurious hybridization signals under our conditions. A ³⁵S-labeled single stranded normal sense riboprobe from the same cDNA construct was used as a negative control. *ltbp-2* expression above background was observed in the snout, base of the skull, tail, paw, lung, vertebrae, and large vessels of mouse embryos. Microscopy of day 16.5 *p.c.* embryo tissue sections, taken from the same slide used to prepare whole mount sections shown in FIG. 12A, demonstrated that the pattern of hybridization was due to significant *ltbp-2* gene expression by perichondrial and vascular wall cells. Positive signals were detected, for example, in perichondrial cells of cartilage aggregates located in the vertebral column (v), forelimb and tail, and at the base of the skull (FIG. 12B). Indeed, the perichondrium (pc) of all cartilage aggregates observed in these mouse embryo tissue sections was positively hybridized. *ltbp-2* was also expressed by vascular wall cells of the aorta (ao), and in blood vessels within lung parenchyma and within the connective tissue supporting hair follicle structures associated with the snout (s). In contrast, *ltbp-2* was expressed at insignificant levels (*i.e.*, below the experimental background) in the generalized mesenchyme/connective tissue, brain, peripheral nerve, tooth rudiment, lung epithelium, cardiac and skeletal muscle, gut epithelium, liver parenchyma, pancreas epithelium and islets of Langerhans, brown fat cells, and kidney parenchyma. These *in situ* hybridization results, which were reproduced using independent tissue sections, demonstrate for the first time that *ltbp-2* expression in developing mouse tissues is more restricted than that of *ltbp-3*.

3. Chromosomal Localization of Mouse *ltbp-2* Gene

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The murine *ltbp-2* gene was assigned to a mouse chromosome by PCR™ analysis of genomic DNA from a mapping panel consisting of 19 mouse x Chinese hamster and 1 mouse x rat somatic cell hybrid lines as described (Li *et al.*, 1995). A PCR™ product of the expected size (600 bp) was obtained from hybrid cells that had retained mouse chromosome 12. All other mouse chromosomes (except chromosome 12) were excluded by at least four discordant hybrids. Fluorescent in situ hybridization using murine *ltbp-2* genomic and cDNA probes generated identical results that localized the *ltbp-2* gene to mouse chromosome 12, band D. 14/20 metaphase spreads analyzed exhibited a fluorescent signal on both chromatids of chromosome 12 at the band D site, and 10/12 had signals on both chromosome 12 homologs. No specific signals were seen on other chromosomes. This region in the mouse is a conserved syntenic region with human chromosome 14, band 14q24, the site of the human *LTBP-2* genetic locus (Moren *et al.*, 1994), thereby providing strong support for the notion that the murine *ltbp-2* gene is the true homolog of human *LTBP-2*.

EXAMPLE IV

EXPRESSION OF RECOMBINANT LTBP PROTEIN

The *Pichia* Expression Kit (Invitrogen, Inc.) may be used to prepare recombinant LTBP protein. This kit, based on the methylotrophic yeast, *Pichia pastoris*, allows high-level expression of recombinant protein in an easy-to-use relatively inexpensive system. In the absence of the preferred carbon source, glucose, *P. pastoris* utilizes methanol as a carbon source. The *AOX1* promoter controls the gene that codes for the expression of the enzyme alcohol oxidase, which catalyzes the first step in the metabolism of methanol. This promoter, which is induced by methanol, has been characterized and incorporated into a series of *Pichia* expression vectors. This feature of *Pichia* has been exploited to express high levels of recombinant proteins often in the range of grams per liter. Because it is eukaryotic, *P. pastoris* utilizes posttranslational modification pathways that are similar to those used by mammalian cells. This implies that the recombinant LTBP-2 or LTBP-3 protein will be glycosylated and will contain disulfide bonds.

For preparation of a recombinant LTBP-2 or LTBP-3 protein, the native LTBP-2 or LTBP-3 cDNA is modified by the addition of a commercially available epitope tag (the HA epitope, Pharmacia, LKB Biotechnology, Inc.). Such fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, by application of nucleic acid reproduction technology, such as the PCR™ technology of U.S. Patent 4,683,202 (herein incorporated by reference) or by introducing selected sequences into recombinant vectors for recombinant production. (PCR™ is a registered trademark of Hoffmann-La Roche, Inc.). This is followed by cloning into the *Pichia* expression vector. The resulting plasmid is characterized by DNA sequence analysis, linearized by digestion with *NotI*, and spheroplasts will be prepared and transformed with the linearized construct according to the manufacturer's recommendations.

Transformation facilitates a recombination event *in vivo* between the 5' and 3' *AOXI* sequences in the *Pichia* vector and those in the *Pichia* genome. The result is the replacement of *AOXI* with the gene of interest.

Transformants are then plated on histidine-deficient media, which will select for successfully transformed cells. Transformants are further selected against slow growth on growth media containing methanol. Positive transformants are grown for 2 days in liquid culture and then for 2-6 days in broth that uses methanol as the sole carbon source. Protein expression is evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western hybridization using a commercially available polyclonal antisera to the HA epitope (Pharmacia). Recombinant LTBP-2 or LTBP-3 protein may be purified according to the manufacturer's recommendations, dialyzed against double distilled, deionized water and lyophilized in 10 mg aliquots.

* * * * *

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the composition, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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The following literature citations as well as those cited above are incorporated in pertinent part by reference herein for the reasons cited in the above text.

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SEQUENCE LISTING

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(F) ZIP: 77210
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS/ASCII
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: Unknown
(B) FILING DATE: Concurrently Herewith
(C) CLASSIFICATION: Unknown
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US PCT/US95/02251
(B) FILING DATE: 21-FEB-1995
(C) CLASSIFICATION: Unknown
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/316,650
(B) FILING DATE: 30-SEP-1994
(C) CLASSIFICATION: Unknown
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/199,780
(B) FILING DATE: 18-FEB-1994
(C) CLASSIFICATION: Unknown
- (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Fussey, Shelley P.M.
(B) REGISTRATION NUMBER: 39,458
(C) REFERENCE/DOCKET NUMBER: UMIC:013/MOO
- (ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: (713) 789-2679
(C) TELEX: 79-0924

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5499 base pairs

(B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "DNA"

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..5499

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

15	ATG GAG AGC ACC TCC CCG CGA GGT CTC CGG TGC CCA CAG CTC TGC AGC	48
	Met Glu Ser Thr Ser Pro Arg Gly Leu Arg Cys Pro Gln Leu Cys Ser	
	1 5 10 15	
20	CAC TCT GGC GCC ATG AGA GCG CCG ACC ACC GCT CGC TGC TCC GGA TGC	96
	His Ser Gly Ala Met Arg Ala Pro Thr Thr Ala Arg Cys Ser Gly Cys	
	20 25 30	
25	ATC CAA CGG GTG CGT TGG AGG GGC TTC CTG CCA CTT GTC CTG GCT GTC	144
	Ile Gln Arg Val Arg Trp Arg Gly Phe Leu Pro Leu Val Leu Ala Val	
	35 40 45	
30	TTG ATG GGG ACA AGT CAT GCC CAA CGG GAT TCC ATA GGG AGA TAC GAA	192
	Leu Met Gly Thr Ser His Ala Gln Arg Asp Ser Ile Gly Arg Tyr Glu	
	50 55 60	
35	CCA GCT AGC AGG GAT GCG AAT CGG TTG TGG CAC CCC GTG GGC AGC CAC	240
	Pro Ala Ser Arg Asp Ala Asn Arg Leu Trp His Pro Val Gly Ser His	
	65 70 75 80	
40	CCC GCA GCG GCT GCA GCC AAG GTG TAC AGT CTG TTC CGA GAG CCT GAC	288
	Pro Ala Ala Ala Ala Ala Lys Val Tyr Ser Leu Phe Arg Glu Pro Asp	
	85 90 95	
45	GCG CCG GTC CCC GGC TTG TCG CCC TCT GAG TGG AAC CAG CCG GCC CAG	336
	Ala Pro Val Pro Gly Leu Ser Pro Ser Glu Trp Asn Gln Pro Ala Gln	
	100 105 110	
50	GGG AAC CCG GGA TGG CTC GCA GAG GCC GAG GCC AGG AGG CCA CCT CGA	384
	Gly Asn Pro Gly Trp Leu Ala Glu Ala Glu Ala Arg Arg Pro Pro Arg	
	115 120 125	
55	ACC CAG CAG CTG CGT CGA GTC CAG CCA CCT GTC CAG ACT CGG AGA AGC	432
	Thr Gln Gln Leu Arg Arg Val Gln Pro Pro Val Gln Thr Arg Arg Ser	
	130 135 140	
60	CAT CCC CGG GGC CAG CAG CAG ATA GCA GCC CGG GCT GCA CCT TCT GTC	480
	His Pro Arg Gly Gln Gln Gln Ile Ala Ala Arg Ala Ala Pro Ser Val	
	145 150 155 160	
65	GCG CGC CTG GAA ACC CCT CAG CGA CCC GCG GCT GCA CGG CGA GGG CGG	528
	Ala Arg Leu Glu Thr Pro Gln Arg Pro Ala Ala Ala Arg Arg Gly Arg	
	165 170 175	
70	CTC ACT GGG AGA AAT GTC TGC GGG GGA CAG TGC TGC CCA GGA TGG ACA	576
	Leu Thr Gly Arg Asn Val Cys Gly Gly Gln Cys Cys Pro Gly Trp Thr	
	180 185 190	
75	ACA TCA AAC AGC ACC AAC CAC TGT ATC AAA CCT GTG TGT CAG CCT CCC	624

	Thr	Ser	Asn	Ser	Thr	Asn	His	Cys	Ile	Lys	Pro	Val	Cys	Gln	Pro	Pro	
			195					200					205				
5	TGT	CAG	AAC	CGA	GGC	TCC	TGC	AGC	AGG	CCC	CAG	GTC	TGC	ATC	TGC	CGT	672
	Cys	Gln	Asn	Arg	Gly	Ser	Cys	Ser	Arg	Pro	Gln	Val	Cys	Ile	Cys	Arg	
			210				215					220					
10	TCT	GGC	TTC	CGT	GGG	GCG	CGC	TGT	GAG	GAG	GTC	ATC	CCT	GAG	GAG	GAA	720
	Ser	Gly	Phe	Arg	Gly	Ala	Arg	Cys	Glu	Glu	Val	Ile	Pro	Glu	Glu	Glu	
						230					235					240	
15	TTT	GAC	CCT	CAG	AAT	GCC	AGG	CCT	GTG	CCC	AGA	CGC	TCA	GTG	GAG	AGA	768
	Phe	Asp	Pro	Gln	Asn	Ala	Arg	Pro	Val	Pro	Arg	Arg	Ser	Val	Glu	Arg	
					245					250					255		
20	GCA	CCC	GGT	CCT	CAC	AGA	AGC	AGT	GAG	GCC	AGA	GGA	AGT	CTA	GTG	ACC	816
	Ala	Pro	Gly	Pro	His	Arg	Ser	Ser	Glu	Ala	Arg	Gly	Ser	Leu	Val	Thr	
				260					265					270			
25	AGA	ATA	CAG	CCG	CTG	GTA	CCA	CCA	CCA	TCA	CCA	CCT	CCA	TCT	CGG	CGC	864
	Arg	Ile	Gln	Pro	Leu	Val	Pro	Pro	Pro	Ser	Pro	Pro	Pro	Ser	Arg	Arg	
				275				280					285				
30	CTC	AGC	CAG	CCC	TGG	CCC	CTG	CAG	CAG	CAC	TCA	GGG	CCG	TCC	AGG	ACA	912
	Leu	Ser	Gln	Pro	Trp	Pro	Leu	Gln	Gln	His	Ser	Gly	Pro	Ser	Arg	Thr	
				290			295					300					
35	GTT	CGT	CGG	TAT	CCG	GCC	ACT	GGT	GCC	AAT	GGC	CAG	CTG	ATG	TCC	AAC	960
	Val	Arg	Arg	Tyr	Pro	Ala	Thr	Gly	Ala	Asn	Gly	Gln	Leu	Met	Ser	Asn	
						310					315					320	
40	GCT	TTG	CCT	TCA	GGA	CTC	GAG	CTG	AGA	GAC	AGC	AGC	CCA	CAG	GCA	GCA	1008
	Ala	Leu	Pro	Ser	Gly	Leu	Glu	Leu	Arg	Asp	Ser	Ser	Pro	Gln	Ala	Ala	
					325					330					335		
45	CAT	GTG	AAC	CAT	CTC	TCA	CCC	CCC	TGG	GGG	CTG	AAC	CTC	ACC	GAG	AAA	1056
	His	Val	Asn	His	Leu	Ser	Pro	Pro	Trp	Gly	Leu	Asn	Leu	Thr	Glu	Lys	
				340					345					350			
50	ATC	AAG	AAA	ATC	AAA	GTC	GTC	TTC	ACC	CCC	ACC	ATC	TGC	AAG	CAG	ACC	1104
	Ile	Lys	Lys	Ile	Lys	Val	Val	Phe	Thr	Pro	Thr	Ile	Cys	Lys	Gln	Thr	
				355				360					365				
55	TGT	GCC	CGG	GGA	CGC	TGT	GCC	AAC	AGC	TGT	GAG	AAG	GGT	GAC	ACC	ACC	1152
	Cys	Ala	Arg	Gly	Arg	Cys	Ala	Asn	Ser	Cys	Glu	Lys	Gly	Asp	Thr	Thr	
				370			375					380					
60	ACC	TTG	TAC	AGT	CAG	GGT	GGC	CAT	GGG	CAT	GAC	CCC	AAG	TCT	GGC	TTC	1200
	Thr	Leu	Tyr	Ser	Gln	Gly	Gly	His	Gly	His	Asp	Pro	Lys	Ser	Gly	Phe	
						390					395					400	
65	CGT	ATC	TAT	TTC	TGC	CAA	ATC	CCC	TGC	CTG	AAT	GGT	GGC	CGC	TGC	ATC	1248
	Arg	Ile	Tyr	Phe	Cys	Gln	Ile	Pro	Cys	Leu	Asn	Gly	Gly	Arg	Cys	Ile	
					405					410					415		
70	GGC	CGG	GAC	GAG	TGC	TGG	TGT	CCA	GCC	AAC	TCC	ACA	GGA	AAG	TTC	TGC	1296
	Gly	Arg	Asp	Glu	Cys	Trp	Cys	Pro	Ala	Asn	Ser	Thr	Gly	Lys	Phe	Cys	
				420					425					430			
75	CAT	CTG	CCT	GTC	CCG	CAG	CCA	GAC	AGG	GAA	CCT	GCA	GGG	CGA	GGT	TCC	1344
	His	Leu	Pro	Val	Pro	Gln	Pro	Asp	Arg	Glu	Pro	Ala	Gly	Arg	Gly	Ser	
				435				440					445				

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	CGG	CAC	AGA	ACC	CTG	CTG	GAA	GGT	CCC	CTG	AAG	CAA	TCC	ACC	TTC	ACG	1392
	Arg	His	Arg	Thr	Leu	Leu	Glu	Gly	Pro	Leu	Lys	Gln	Ser	Thr	Phe	Thr	
	450						455					460					
5	CTG	CCT	CTC	TCT	AAC	CAG	CTC	GCC	TCT	GTG	AAC	CCC	TCG	CTG	GTG	AAG	1440
	Leu	Pro	Leu	Ser	Asn	Gln	Leu	Ala	Ser	Val	Asn	Pro	Ser	Leu	Val	Lys	
	465					470					475					480	
10	GTG	CAA	ATT	CAT	CAC	CCG	CCT	GAG	GCC	TCT	GTG	CAG	ATT	CAC	CAG	GTG	1488
	Val	Gln	Ile	His	His	Pro	Pro	Glu	Ala	Ser	Val	Gln	Ile	His	Gln	Val	
					485					490					495		
15	GCC	CGG	GTC	CGG	GGT	GAG	CTG	GAC	CCC	GTG	CTG	GAG	GAC	AAC	AGT	GTG	1536
	Ala	Arg	Val	Arg	Gly	Glu	Leu	Asp	Pro	Val	Leu	Glu	Asp	Asn	Ser	Val	
				500					505					510			
20	GAG	ACC	AGA	GCC	TCT	CAT	CGC	CCC	CAC	GGC	AAC	CTA	GGC	CAC	AGC	CCC	1584
	Glu	Thr	Arg	Ala	Ser	His	Arg	Pro	His	Gly	Asn	Leu	Gly	His	Ser	Pro	
			515					520					525				
	TGG	GCC	AGC	AAC	AGC	ATA	CCC	GCT	CGG	GCC	GGA	GAG	GCC	CCT	CGG	CCA	1632
	Trp	Ala	Ser	Asn	Ser	Ile	Pro	Ala	Arg	Ala	Gly	Glu	Ala	Pro	Arg	Pro	
	530						535					540					
25	CCA	CCA	GTG	CTG	TCT	AGG	CAT	TAT	GGA	CTT	CTG	GGC	CAG	TGT	TAC	CTG	1680
	Pro	Pro	Val	Leu	Ser	Arg	His	Tyr	Gly	Leu	Leu	Gly	Gln	Cys	Tyr	Leu	
	545					550					555					560	
30	AGC	ACG	GTG	AAT	GGA	CAG	TGT	GCT	AAC	CCC	CTA	GGT	AGT	CTG	ACT	TCT	1728
	Ser	Thr	Val	Asn	Gly	Gln	Cys	Ala	Asn	Pro	Leu	Gly	Ser	Leu	Thr	Ser	
					565					570					575		
35	CAG	GAG	GAC	TGC	TGT	GGC	AGT	GTG	GGG	ACC	TTC	TGG	GGG	GTG	ACC	TCC	1776
	Gln	Glu	Asp	Cys	Cys	Gly	Ser	Val	Gly	Thr	Phe	Trp	Gly	Val	Thr	Ser	
				580					585					590			
40	TGT	GCT	CCC	TGC	CCA	CCC	AGA	CAA	GAG	GGT	CCA	GCC	TTC	CCA	GTG	ATT	1824
	Cys	Ala	Pro	Cys	Pro	Pro	Arg	Gln	Glu	Gly	Pro	Ala	Phe	Pro	Val	Ile	
			595				600						605				
45	GAA	AAT	GGC	CAG	CTG	GAG	TGT	CCC	CAA	GGA	TAC	AAG	AGA	CTG	AAC	CTC	1872
	Glu	Asn	Gly	Gln	Leu	Glu	Cys	Pro	Gln	Gly	Tyr	Lys	Arg	Leu	Asn	Leu	
		610				615						620					
50	AGC	CAC	TGC	CAA	GAT	ATC	AAT	GAG	TGC	CTG	ACC	CTG	GGC	CTC	TGC	AAG	1920
	Ser	His	Cys	Gln	Asp	Ile	Asn	Glu	Cys	Leu	Thr	Leu	Gly	Leu	Cys	Lys	
	625					630				635						640	
55	GAC	TCG	GAG	TGC	GTG	AAC	ACC	AGG	GGC	AGC	TAC	CTG	TGC	ACC	TGC	AGG	1968
	Asp	Ser	Glu	Cys	Val	Asn	Thr	Arg	Gly	Ser	Tyr	Leu	Cys	Thr	Cys	Arg	
					645					650					655		
60	CCT	GGC	CTC	ATG	CTG	GAT	CCG	TCA	AGG	AGC	CGC	TGC	GTA	TCG	GAC	AAG	2016
	Pro	Gly	Leu	Met	Leu	Asp	Pro	Ser	Arg	Ser	Arg	Cys	Val	Ser	Asp	Lys	
				660					665					670			
	GCT	GTC	TCC	ATG	CAG	CAG	GGA	CTA	TGC	TAC	CGG	TCA	CTG	GGG	TCT	GGT	2064
	Ala	Val	Ser	Met	Gln	Gln	Gly	Leu	Cys	Tyr	Arg	Ser	Leu	Gly	Ser	Gly	
			675				680						685				
60	ACC	TGC	ACC	CTG	CCT	TTG	GTT	CAT	CGG	ATC	ACC	AAG	CAG	ATA	TGC	TGC	2112
	Thr	Cys	Thr	Leu	Pro	Leu	Val	His	Arg	Ile	Thr	Lys	Gln	Ile	Cys	Cys	

	690					695					700						
5	TGC Cys 705	AGC Ser	CGT Arg	GTG Val	GGC Gly 710	AAA Lys	GCC Ala	TGG Trp	GGT Gly	AGC Ser	ACA Thr 715	TGT Cys	GAA Glu	CAG Gln	TGT Cys	CCC Pro 720	2160
10	CTG Leu	CCT Pro	GGC Gly	ACA Thr 725	GAA Glu	GCC Ala	TTC Phe	AGG Arg	GAG Glu	ATC Ile 730	TGC Cys	CCT Pro	GCT Ala	GGC Gly 735	CAT His	GGC Gly	2208
15	TAC Tyr	ACC Thr	TAC Tyr 740	TCG Ser	AGC Ser	TCA Ser	GAC Asp	ATC Ile	CGC Arg 745	CTG Leu	TCT Ser	ATG Met	AGG Arg 750	AAA Lys	GCC Ala	GAA Glu	2256
20	GAA Glu	GAG Glu	GAA Glu 755	CTG Leu	GCT Ala	AGC Ser	CCC Pro	TTA Leu 760	AGG Arg	GAG Glu	CAG Gln	ACA Thr 765	GAG Glu	CAG Gln	AGC Ser	ACT Thr	2304
25	GCA Ala	CCC Pro 770	CCA Pro	CCT Pro	GGG Gly	CAA Gln 775	GCA Ala	GAG Glu 775	AGG Arg	CAA Gln	CCA Pro	CTC Leu 780	CGG Arg	GCA Ala	GCC Ala	ACC Thr	2352
30	GCC Ala 785	ACC Thr	TGG Trp	ATT Ile	GAG Glu 790	GCT Ala	GAG Glu	ACC Thr	CTC Leu	CCT Pro	GAC Asp 795	AAA Lys	GGT Gly	GAC Asp	TCT Ser	CGG Arg 800	2400
35	GCT Ala	GTT Val	CAG Gln	ATC Ile 805	ACA Thr	ACC Thr	AGT Ser	GCT Ala	CCC Pro	CAC His 810	CTA Leu	CCT Pro	GCC Ala	CGG Arg	GTA Val 815	CCA Pro	2448
40	GGG Gly	GAT Asp	GCC Ala	ACT Thr 820	GGA Gly	AGA Arg	CCA Pro	GCA Ala	CCA Pro	TCC Ser	TTG Leu	CCT Pro	GGA Gly	CAG Gln 830	GGC Gly	ATT Ile	2496
45	CCA Pro	GAG Glu	AGT Ser 835	CCA Pro	GCA Ala	GAA Glu	GAG Glu 840	CAA Gln	GTG Val	ATT Ile	CCC Pro	TCC Ser	AGT Ser 845	GAT Asp	GTC Val	TTG Leu	2544
50	GTG Val	ACA Thr 850	CAC His	AGC Ser	CCC Pro	CCA Pro	GAC Asp 855	TTT Phe	GAT Asp	CCA Pro	TGT Cys	TTT Phe 860	GCT Ala	GGA Gly	GCC Ala	TCC Ser	2592
55	AAC Asn 865	ATC Ile	TGT Cys	GGC Gly	CCT Pro	GGG Gly 870	ACC Thr	TGT Cys	GTG Val	AGC Ser	CTC Leu 875	CCA Pro	AAT Asn	GGA Gly	TAC Tyr	AGA Arg 880	2640
60	TGT Cys	GTC Val	TGC Cys	AGC Ser	CCT Pro 885	GGC Gly	TAC Tyr	CAG Gln	CTA Leu	CAC His 890	CCC Pro	AGC Ser	CAA Gln	GAC Asp	TAC Tyr 895	TGT Cys	2688
65	ACT Thr	GAT Asp	GAC Asp	AAC Asn 900	GAG Glu	TGT Cys	ATG Met	AGG Arg	AAC Asn 905	CCC Pro	TGT Cys	GAA Glu	GGA Gly	AGA Arg 910	GGG Gly	CGC Arg	2736
70	TGT Cys	GTC Val	AAC Asn 915	AGT Ser	GTG Val	GGC Gly	TCC Ser	TAC Tyr 920	TCC Ser	TGC Cys	CTC Leu	TGC Cys	TAT Tyr 925	CCT Pro	GGC Gly	TAC Tyr	2784
75	ACA Thr 930	CTA Leu	GTC Val	ACC Thr	CTC Leu	GGA Gly 935	GAC Asp	ACA Thr	CAG Gln	GAG Glu	TGC Cys	CAA Gln 940	GAT Asp	ATC Ile	GAT Asp	GAG Glu	2832

		TGT	GAG	CAG	CCC	GGG	GTG	TGC	AGT	GGT	GGG	CGA	TGC	AGC	AAC	ACG	GAG	2880
		Cys	Glu	Gln	Pro	Gly	Val	Cys	Ser	Gly	Gly	Arg	Cys	Ser	Asn	Thr	Glu	
		945					950					955					960	
5		GGC	TCG	TAC	CAC	TGC	GAG	TGT	GAT	CGG	GGC	TAC	ATC	ATG	GTC	AGG	AAA	2928
		Gly	Ser	Tyr	His	Cys	Glu	Cys	Asp	Arg	Gly	Tyr	Ile	Met	Val	Arg	Lys	
						965					970					975		
10		GGA	CAC	TGT	CAA	GAT	ATC	AAC	GAA	TGC	CGT	CAC	CCT	GGT	ACC	TGC	CCT	2976
		Gly	His	Cys	Gln	Asp	Ile	Asn	Glu	Cys	Arg	His	Pro	Gly	Thr	Cys	Pro	
					980					985					990			
15		GAT	GGG	AGA	TGC	GTC	AAC	TCC	CCT	GGC	TCC	TAC	ACT	TGT	CTG	GCC	TGT	3024
		Asp	Gly	Arg	Cys	Val	Asn	Ser	Pro	Gly	Ser	Tyr	Thr	Cys	Leu	Ala	Cys	
				995					1000					1005				
20		GAG	GAG	GGC	TAT	GTA	GGC	CAG	AGT	GGG	AGC	TGT	GTA	GAT	GTC	AAT	GAG	3072
		Glu	Glu	Gly	Tyr	Val	Gly	Gln	Ser	Gly	Ser	Cys	Val	Asp	Val	Asn	Glu	
		1010					1015						1020					
25		TGT	CTG	ACC	CCT	GGG	ATA	TGT	ACC	CAT	GGA	AGG	TGC	ATC	AAC	ATG	GAA	3120
		Cys	Leu	Thr	Pro	Gly	Ile	Cys	Thr	His	Gly	Arg	Cys	Ile	Asn	Met	Glu	
		1025				1030					1035						1040	
30		GGC	TCC	TTT	AGA	TGC	TCC	TGT	GAG	CCG	GGC	TAT	GAG	GTC	ACC	CCA	GAC	3168
		Gly	Ser	Phe	Arg	Cys	Ser	Cys	Glu	Pro	Gly	Tyr	Glu	Val	Thr	Pro	Asp	
						1045					1050					1055		
35		AAG	AAG	GGC	TGC	CGA	GAT	GTG	GAC	GAG	TGT	GCC	AGC	CGA	GCC	TCG	TGC	3216
		Lys	Lys	Gly	Cys	Arg	Asp	Val	Asp	Glu	Cys	Ala	Ser	Arg	Ala	Ser	Cys	
					1060					1065					1070			
40		CCC	ACG	GGC	CTC	TGC	CTC	AAC	ACG	GAG	GGC	TCC	TTC	ACC	TGC	TCA	GCC	3264
		Pro	Thr	Gly	Leu	Cys	Leu	Asn	Thr	Glu	Gly	Ser	Phe	Thr	Cys	Ser	Ala	
				1075				1080						1085				
45		TGT	CAG	AGC	GGG	TAC	TGG	GTG	AAC	GAA	GAT	GGC	ACT	GCC	TGT	GAA	GAC	3312
		Cys	Gln	Ser	Gly	Tyr	Trp	Val	Asn	Glu	Asp	Gly	Thr	Ala	Cys	Glu	Asp	
		1090					1095						1100					
50		TTG	GAT	GAA	TGT	GCC	TTC	CCT	GGA	GTC	TGC	CCC	ACA	GGC	GTC	TGC	ACC	3360
		Leu	Asp	Glu	Cys	Ala	Phe	Pro	Gly	Val	Cys	Pro	Thr	Gly	Val	Cys	Thr	
		1105				1110						1115					1120	
55		AAT	ACT	GTA	GGC	TCC	TTC	TCC	TGC	AAG	GAC	TGT	GAC	CAG	GGC	TAC	CGG	3408
		Asn	Thr	Val	Gly	Ser	Phe	Ser	Cys	Lys	Asp	Cys	Asp	Gln	Gly	Tyr	Arg	
					1125					1130						1135		
60		CCC	AAC	CCC	CTG	GGC	AAC	AGA	TGC	GAA	GAT	GTG	GAT	GAG	TGT	GAA	GGT	3456
		Pro	Asn	Pro	Leu	Gly	Asn	Arg	Cys	Glu	Asp	Val	Asp	Glu	Cys	Glu	Gly	
					1140					1145					1150			
55		CCC	CAA	AGC	AGC	TGC	CGG	GGA	GGC	GAA	TGC	AAG	AAC	ACA	GAA	GGT	TCC	3504
		Pro	Gln	Ser	Ser	Cys	Arg	Gly	Gly	Glu	Cys	Lys	Asn	Thr	Glu	Gly	Ser	
				1155				1160						1165				
60		TAC	CAA	TGC	CTC	TGT	CAC	CAG	GGC	TTC	CAG	CTG	GTC	AAT	GGC	ACC	ATG	3552
		Tyr	Gln	Cys	Leu	Cys	His	Gln	Gly	Phe	Gln	Leu	Val	Asn	Gly	Thr	Met	
		1170					1175						1180					
60		TGT	GAG	GAC	GTG	AAT	GAG	TGT	GTT	GGG	GAA	GAG	CAT	TGT	GCT	CCT	CAC	3600
		Cys	Glu	Asp	Val	Asn	Glu	Cys	Val	Gly	Glu	Glu	His	Cys	Ala	Pro	His	

60

1185																1190																1195																1200	
GGC Gly	GAG Glu	TGC Cys	CTC Leu	AAC Asn	AGC Ser	CTG Leu	GGC Gly	TCC Ser	TTC Phe	TTC Phe	TGC Cys	CTC Leu	TGT Cys	GCA Ala	CCC Pro	3648																																	
				1205							1210							1215																															
GGC Gly	TTT Phe	GCT Ala	AGT Ser	GCT Ala	GAG Glu	GGG Gly	GGC Gly	ACC Thr	AGA Arg	TGC Cys	CAG Gln	GAT Asp	GTT Val	GAT Asp	GAA Glu	3696																																	
				1220							1225							1230																															
TGT Cys	GCA Ala	GCC Ala	ACA Thr	GAC Asp	CCG Pro	TGT Cys	CCG Pro	GGA Gly	GGA Gly	CAC His	TGT Cys	GTC Val	AAC Asn	ACA Thr	GAG Glu	3744																																	
				1235							1240							1245																															
GGC Gly	TCC Ser	TTC Phe	AGC Ser	TGT Cys	CTG Leu	TGT Cys	GAG Glu	ACT Thr	GCT Ala	TCC Ser	TTC Phe	CAG Gln	CCC Pro	TCC Ser	CCA Pro	3792																																	
				1250							1255							1260																															
GAC Asp	AGC Ser	GGA Gly	GAA Glu	TGT Cys	TTG Leu	GAT Asp	ATT Ile	GAT Asp	GAG Glu	TGT Cys	GAG Glu	GAC Asp	CGT Arg	GAA Glu	GAC Asp	3840																																	
				1265							1270							1280																															
CCG Pro	GTG Val	TGC Cys	GGA Gly	GCC Ala	TGG Trp	AGG Arg	TGT Cys	GAG Glu	AAC Asn	AGT Ser	CCT Pro	GGT Gly	TCC Ser	TAC Tyr	CGC Arg	3888																																	
				1285							1290							1295																															
TGC Cys	ATC Ile	CTG Leu	GAC Asp	TGC Cys	CAG Gln	CCT Pro	GGA Gly	TTC Phe	TAT Tyr	GTG Val	GCG Ala	CCA Pro	AAT Asn	GGA Gly	GAC Asp	3936																																	
				1300							1305							1310																															
TGC Cys	ATT Ile	GAC Asp	ATA Ile	GAT Asp	GAA Glu	TGT Cys	GCC Ala	AAT Asn	GAC Asp	ACT Thr	GTG Val	TGT Cys	GGG Gly	AAC Asn	CAT His	3984																																	
				1315							1320							1325																															
GGC Gly	TTC Phe	TGT Cys	GAC Asp	AAC Asn	ACG Thr	GAC Asp	GGC Gly	TCC Ser	TTC Phe	CGC Arg	TGC Cys	CTG Leu	TGT Cys	GAC Asp	CAG Gln	4032																																	
				1330							1335							1340																															
GGC Gly	TTC Phe	GAG Glu	ACC Thr	TCA Ser	CCA Pro	TCA Ser	GGC Gly	TGG Trp	GAG Glu	TGT Cys	GTT Val	GAT Asp	GTG Val	AAC Asn	GAG Glu	4080																																	
				1345							1350							1360																															
TGT Cys	GAG Glu	CTC Leu	ATG Met	ATG Met	GCA Ala	GTG Val	TGT Cys	GGG Gly	GAT Asp	GCG Ala	CTC Leu	TGT Cys	GAG Glu	AAC Asn	GTG Val	4128																																	
				1365							1370							1375																															
GAA Glu	GGC Gly	TCC Ser	TTC Phe	CTG Leu	TGC Cys	CTT Leu	TGC Cys	GCC Ala	AGT Ser	GAC Asp	CTT Leu	GAG Glu	GAG Glu	TAC Tyr	GAC Asp	4176																																	
				1380							1385							1390																															
GCA Ala	GAA Glu	GAA Glu	GGA Gly	CAC His	TGC Cys	CGT Arg	CCT Pro	CGG Arg	GTG Val	GCT Ala	GGA Gly	GCT Ala	CAG Gln	AGA Arg	ATC Ile	4224																																	
				1395							1400							1405																															
CCA Pro	GAG Glu	GTC Val	CGG Arg	ACA Thr	GAG Glu	GAC Asp	CAG Gln	GCT Ala	CCA Pro	AGC Ser	CTT Leu	ATC Ile	CGC Arg	ATG Met	GAA Glu	4272																																	
				1410							1415							1420																															
TGC Cys	TAC Tyr	TCT Ser	GAA Glu	CAC His	AAT Asn	GGT Gly	GGT Gly	CCT Pro	CCC Pro	TGC Cys	TCT Ser	CAA Gln	ATC Ile	CTG Leu	GGC Gly	4320																																	
				1425							1430							1440																															
											1435																																						

CAG Gln	AAC Asn	TCC Ser	ACA Thr	CAG Gln 1445	GCC Ala	GAG Glu	TGC Cys	TGC Cys	TGC Cys 1450	ACT Thr	CAG Gln	GGT Gly	GCC Ala	AGA Arg 1455	TGG Trp	4368
GGA Gly	AAG Lys	GCC Ala	TGT Cys 1460	GCG Ala	CCC Pro	TGC Cys	CCA Pro	TCT Ser 1465	GAG Glu	GAC Asp	TCA Ser	GTT Val 1470	GAA Glu	TTC Phe	AGT Ser	4416
CAG Gln	CTC Leu	TGC Cys 1475	CCC Pro	AGT Ser	GGT Gly	CAA Gln	GGT Gly 1480	TAC Tyr	ATC Ile	CCA Pro	GTG Val 1485	GAA Glu	GGA Gly	GCC Ala	TGG Trp	4464
ACA Thr	TTT Phe 1490	GGA Gly	CA ^A Gln	ACC Thr	ATG Met	TAT Tyr 1495	ACA Thr	GAT Asp	GCC Ala	GAT Asp	GAA Glu 1500	TGT Cys	GTA Val	CTG Leu	TTT Phe	4512
GGG Gly 1505	CCT Pro	GCT Ala	CTC Leu	TGC Cys	CAG Gln 1510	AAT Asn	GGC Gly	CGA Arg	TGC Cys 1515	TCA Ser	AAC Asn	ATA Ile	GTG Val	CCT Pro	GGC Gly 1520	4560
TAC Tyr	ATT Ile	TGC Cys	CTG Leu	TGC Cys 1525	AAC Asn	CCT Pro	GGC Gly	TAC Tyr	CAC His 1530	TAT Tyr	GAT Asp	GCC Ala	TCC Ser	AGC Ser 1535	AGG Arg	4608
AAG Lys	TGC Cys	CAG Gln 1540	GAT Asp	CAC His	AAC Asn	GAA Glu	TGC Cys 1545	CAG Gln	GAC Asp	TTG Leu	GCC Ala	TGT Cys 1550	GAG Glu	AAC Asn	GGT Gly	4656
GAG Glu	TGT Cys 1555	GTG Val	AAC Asn	CAA Gln	GAA Glu	GGC Gly 1560	TCC Ser	TTC Phe	CAT His	TGC Cys 1565	CTC Leu	TGC Cys	AAT Asn	CCC Pro	CCC Pro	4704
CTC Leu	ACC Thr 1570	CTA Leu	GAC Asp	CTC Leu	AGT Ser	GGG Gly 1575	CAG Gln	CGC Arg	TGT Cys	GTG Val 1580	AAC Asn	ACG Thr	ACC Thr	AGC Ser	AGC Ser	4752
ACG Thr 1585	GAG Glu	GAC Asp	TTC Phe	CCT Pro	GAC Asp 1590	CAT His	GAC Asp	ATC Ile	CAC His	ATG Met 1595	GAC Asp	ATC Ile	TGC Cys	TGG Trp	AAA Lys 1600	4800
AAA Lys	GTC Val	ACC Thr	AAT Asn 1605	GAT Asp	GTG Val	TGC Cys	AGC Ser	CAG Gln	CCC Pro 1610	TTG Leu	CGT Arg	GGG Gly	CAC His	CAT His 1615	ACC Thr	4848
ACC Thr	TAT Tyr	ACA Thr 1620	GAA Glu	TGC Cys	TGC Cys	TGC Cys	CAA Gln	GAT Asp 1625	GGG Gly	GAG Glu	GCC Ala	TGG Trp 1630	AGC Ser	CAG Gln	CAA Gln	4896
TGC Cys	GCT Ala 1635	CTG Leu	TGC Cys	CCG Pro	CCC Pro	AGG Arg	AGC Ser	TCT Ser 1640	GAG Glu	GTC Val	TAC Tyr 1645	GCT Ala	CAG Gln	CTG Leu	TGC Cys	4944
AAC Asn 1650	GTG Val	GCT Ala	CGG Arg	ATT Ile	GAG Glu	GCA Ala 1655	GAG Glu	CGC Arg	GGA Gly	GCA Ala 1660	GGG Gly	ATC Ile	CAC His	TTC Phe	CGG Arg	4992
CCA Pro 1665	GGC Gly	TAT Tyr	GAG Glu	TAT Tyr	GGC Gly 1670	CCT Pro	GGC Gly	CTG Leu	GAC Asp 1675	GAT Asp	CTG Leu	CCT Pro	GAA Glu	AAC Asn	CTC Leu 1680	5040
TAC Tyr	GGC Gly	CCA Pro	GAT Asp	GGG Gly	GCT Ala	CCC Pro	TTC Phe	TAT Tyr	AAC Asn	TAC Tyr	CTA Leu	GGC Gly	CCC Pro	GAG Glu	GAC Asp	5088

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	1685	1690	1695	
	ACT GCC CCT GAG CCT CCC TTC TCC AAC CCA GCC AGC CAG CCG GGA GAC			5136
	Thr Ala Pro Glu Pro Pro Phe Ser Asn Pro Ala Ser Gln Pro Gly Asp			
	1700	1705	1710	
	AAC ACA CCT GTC CTT GAG CCT CCT CTG CAG CCC TCT GAA CTT CAG CCT			5184
	Asn Thr Pro Val Leu Glu Pro Pro Leu Gln Pro Ser Glu Leu Gln Pro			
	1715	1720	1725	
	CAC TAT CTA GCC AGC CAC TCA GAA CCC CCT GCC TCC TTC GAA GGC CTT			5232
	His Tyr Leu Ala Ser His Ser Glu Pro Pro Ala Ser Phe Glu Gly Leu			
	1730	1735	1740	
	CAG GCT GAG GAA TGT GGC ATC CTG AAT GGC TGT GAG AAT GGC CGC TGC			5280
	Gln Ala Glu Glu Cys Gly Ile Leu Asn Gly Cys Glu Asn Gly Arg Cys			
	1745	1750	1755	1760
	GTG CGT GTG CGG GAG GGC TAC ACT TGC GAC TGC TTT GAG GGC TTC CAG			5328
	Val Arg Val Arg Glu Gly Tyr Thr Cys Asp Cys Phe Glu Gly Phe Gln			
	1765	1770	1775	
	CTG GAT GCG CCC ACA TTG GCC TGT GTG GAT GTG AAC GAG TGT GAA GAC			5376
	Leu Asp Ala Pro Thr Leu Ala Cys Val Asp Val Asn Glu Cys Glu Asp			
	1780	1785	1790	
	TTG AAC GGG CCT GCA CGA CTC TGT GCA CAC GGT CAC TGT GAG AAC ACA			5424
	Leu Asn Gly Pro Ala Arg Leu Cys Ala His Gly His Cys Glu Asn Thr			
	1795	1800	1805	
	GAG GGT TCC TAT CGC TGC CAC TGT TCG CCA GGT TAC GTG GCA GAG CCA			5472
	Glu Gly Ser Tyr Arg Cys His Cys Ser Pro Gly Tyr Val Ala Glu Pro			
	1810	1815	1820	
	GGC CCC CCA CAC TGT GCG GCC AAG GAG			5499
	Gly Pro Pro His Cys Ala Ala Lys Glu			
	1825	1830		
	(2) INFORMATION FOR SEQ ID NO:2:			
	(i) SEQUENCE CHARACTERISTICS:			
	(A) LENGTH: 1833 amino acids			
	(B) TYPE: amino acid			
	(D) TOPOLOGY: linear			
	(ii) MOLECULE TYPE: protein			
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:			
	Met Glu Ser Thr Ser Pro Arg Gly Leu Arg Cys Pro Gln Leu Cys Ser			
	1 5 10 15			
	His Ser Gly Ala Met Arg Ala Pro Thr Thr Ala Arg Cys Ser Gly Cys			
	20 25 30			
	Ile Gln Arg Val Arg Trp Arg Gly Phe Leu Pro Leu Val Leu Ala Val			
	35 40 45			
	Leu Met Gly Thr Ser His Ala Gln Arg Asp Ser Ile Gly Arg Tyr Glu			
	50 55 60			

	Pro	Ala	Ser	Arg	Asp	Ala	Asn	Arg	Leu	Trp	His	Pro	Val	Gly	Ser	His	
	65					70					75					80	
5	Pro	Ala	Ala	Ala	Ala	Ala	Lys	Val	Tyr	Ser	Leu	Phe	Arg	Glu	Pro	Asp	
					85					90					95		
	Ala	Pro	Val	Pro	Gly	Leu	Ser	Pro	Ser	Glu	Trp	Asn	Gln	Pro	Ala	Gln	
				100					105					110			
10	Gly	Asn	Pro	Gly	Trp	Leu	Ala	Glu	Ala	Glu	Ala	Arg	Arg	Pro	Pro	Arg	
		115						120					125				
	Thr	Gln	Gln	Leu	Arg	Arg	Val	Gln	Pro	Pro	Val	Gln	Thr	Arg	Arg	Ser	
15		130					135					140					
	His	Pro	Arg	Gly	Gln	Gln	Gln	Ile	Ala	Ala	Arg	Ala	Ala	Pro	Ser	Val	
	145					150					155					160	
20	Ala	Arg	Leu	Glu	Thr	Pro	Gln	Arg	Pro	Ala	Ala	Ala	Arg	Arg	Gly	Arg	
				165						170					175		
	Leu	Thr	Gly	Arg	Asn	Val	Cys	Gly	Gly	Gln	Cys	Cys	Pro	Gly	Trp	Thr	
				180					185					190			
25	Thr	Ser	Asn	Ser	Thr	Asn	His	Cys	Ile	Lys	Pro	Val	Cys	Gln	Pro	Pro	
			195					200					205				
	Cys	Gln	Asn	Arg	Gly	Ser	Cys	Ser	Arg	Pro	Gln	Val	Cys	Ile	Cys	Arg	
30		210					215					220					
	Ser	Gly	Phe	Arg	Gly	Ala	Arg	Cys	Glu	Glu	Val	Ile	Pro	Glu	Glu	Glu	
	225					230					235					240	
	Phe	Asp	Pro	Gln	Asn	Ala	Arg	Pro	Val	Pro	Arg	Arg	Ser	Val	Glu	Arg	
35					245					250					255		
	Ala	Pro	Gly	Pro	His	Arg	Ser	Ser	Glu	Ala	Arg	Gly	Ser	Leu	Val	Thr	
				260					265					270			
40	Arg	Ile	Gln	Pro	Leu	Val	Pro	Pro	Pro	Ser	Pro	Pro	Pro	Ser	Arg	Arg	
			275					280					285				
	Leu	Ser	Gln	Pro	Trp	Pro	Leu	Gln	Gln	His	Ser	Gly	Pro	Ser	Arg	Thr	
45		290					295					300					
	Val	Arg	Arg	Tyr	Pro	Ala	Thr	Gly	Ala	Asn	Gly	Gln	Leu	Met	Ser	Asn	
	305					310					315					320	
50	Ala	Leu	Pro	Ser	Gly	Leu	Glu	Leu	Arg	Asp	Ser	Ser	Pro	Gln	Ala	Ala	
					325					330					335		
	His	Val	Asn	His	Leu	Ser	Pro	Pro	Trp	Gly	Leu	Asn	Leu	Thr	Glu	Lys	
				340					345					350			
55	Ile	Lys	Lys	Ile	Lys	Val	Val	Phe	Thr	Pro	Thr	Ile	Cys	Lys	Gln	Thr	
			355					360					365				
	Cys	Ala	Arg	Gly	Arg	Cys	Ala	Asn	Ser	Cys	Glu	Lys	Gly	Asp	Thr	Thr	
		370					375					380					
60	Thr	Leu	Tyr	Ser	Gln	Gly	Gly	His	Gly	His	Asp	Pro	Lys	Ser	Gly	Phe	
	385					390					395					400	

	Arg	Ile	Tyr	Phe	Cys	Gln	Ile	Pro	Cys	Leu	Asn	Gly	Gly	Arg	Cys	Ile	
					405					410					415		
5	Gly	Arg	Asp	Glu	Cys	Trp	Cys	Pro	Ala	Asn	Ser	Thr	Gly	Lys	Phe	Cys	
				420					425					430			
	His	Leu	Pro	Val	Pro	Gln	Pro	Asp	Arg	Glu	Pro	Ala	Gly	Arg	Gly	Ser	
			435					440					445				
10	Arg	His	Arg	Thr	Leu	Leu	Glu	Gly	Pro	Leu	Lys	Gln	Ser	Thr	Phe	Thr	
		450					455					460					
	Leu	Pro	Leu	Ser	Asn	Gln	Leu	Ala	Ser	Val	Asn	Pro	Ser	Leu	Val	Lys	
	465					470					475					480	
15	Val	Gln	Ile	His	His	Pro	Pro	Glu	Ala	Ser	Val	Gln	Ile	His	Gln	Val	
					485					490					495		
	Ala	Arg	Val	Arg	Gly	Glu	Leu	Asp	Pro	Val	Leu	Glu	Asp	Asn	Ser	Val	
20				500					505					510			
	Glu	Thr	Arg	Ala	Ser	His	Arg	Pro	His	Gly	Asn	Leu	Gly	His	Ser	Pro	
			515					520					525				
25	Trp	Ala	Ser	Asn	Ser	Ile	Pro	Ala	Arg	Ala	Gly	Glu	Ala	Pro	Arg	Pro	
		530					535					540					
	Pro	Pro	Val	Leu	Ser	Arg	His	Tyr	Gly	Leu	Leu	Gly	Gln	Cys	Tyr	Leu	
	545					550					555					560	
30	Ser	Thr	Val	Asn	Gly	Gln	Cys	Ala	Asn	Pro	Leu	Gly	Ser	Leu	Thr	Ser	
					565					570					575		
	Gln	Glu	Asp	Cys	Cys	Gly	Ser	Val	Gly	Thr	Phe	Trp	Gly	Val	Thr	Ser	
			580						585					590			
	Cys	Ala	Pro	Cys	Pro	Pro	Arg	Gln	Glu	Gly	Pro	Ala	Phe	Pro	Val	Ile	
			595					600					605				
40	Glu	Asn	Gly	Gln	Leu	Glu	Cys	Pro	Gln	Gly	Tyr	Lys	Arg	Leu	Asn	Leu	
		610					615					620					
	Ser	His	Cys	Gln	Asp	Ile	Asn	Glu	Cys	Leu	Thr	Leu	Gly	Leu	Cys	Lys	
	625					630					635					640	
45	Asp	Ser	Glu	Cys	Val	Asn	Thr	Arg	Gly	Ser	Tyr	Leu	Cys	Thr	Cys	Arg	
					645					650					655		
	Pro	Gly	Leu	Met	Leu	Asp	Pro	Ser	Arg	Ser	Arg	Cys	Val	Ser	Asp	Lys	
50				660					665					670			
	Ala	Val	Ser	Met	Gln	Gln	Gly	Leu	Cys	Tyr	Arg	Ser	Leu	Gly	Ser	Gly	
				675				680					685				
55	Thr	Cys	Thr	Leu	Pro	Leu	Val	His	Arg	Ile	Thr	Lys	Gln	Ile	Cys	Cys	
		690					695					700					
	Cys	Ser	Arg	Val	Gly	Lys	Ala	Trp	Gly	Ser	Thr	Cys	Glu	Gln	Cys	Pro	
	705					710					715					720	
60	Leu	Pro	Gly	Thr	Glu	Ala	Phe	Arg	Glu	Ile	Cys	Pro	Ala	Gly	His	Gly	
					725					730					735		

5 Pro Glu Val Arg Thr Glu Asp Gln Ala Pro Ser Leu Ile Arg Met Glu
 1410 1415 1420
 Cys Tyr Ser Glu His Asn Gly Gly Pro Pro Cys Ser Gln Ile Leu Gly
 1425 1430 1435 1440
 10 Gln Asn Ser Thr Gln Ala Glu Cys Cys Cys Thr Gln Gly Ala Arg Trp
 1445 1450 1455
 Gly Lys Ala Cys Ala Pro Cys Pro Ser Glu Asp Ser Val Glu Phe Ser
 1460 1465 1470
 15 Gln Leu Cys Pro Ser Gly Gln Gly Tyr Ile Pro Val Glu Gly Ala Trp
 1475 1480 1485
 Thr Phe Gly Gln Thr Met Tyr Thr Asp Ala Asp Glu Cys Val Leu Phe
 1490 1495 1500
 20 Gly Pro Ala Leu Cys Gln Asn Gly Arg Cys Ser Asn Ile Val Pro Gly
 1505 1510 1515 1520
 Tyr Ile Cys Leu Cys Asn Pro Gly Tyr His Tyr Asp Ala Ser Ser Arg
 1525 1530 1535
 25 Lys Cys Gln Asp His Asn Glu Cys Gln Asp Leu Ala Cys Glu Asn Gly
 1540 1545 1550
 Glu Cys Val Asn Gln Glu Gly Ser Phe His Cys Leu Cys Asn Pro Pro
 1555 1560 1565
 30 Leu Thr Leu Asp Leu Ser Gly Gln Arg Cys Val Asn Thr Thr Ser Ser
 1570 1575 1580
 35 Thr Glu Asp Phe Pro Asp His Asp Ile His Met Asp Ile Cys Trp Lys
 1585 1590 1595 1600
 Lys Val Thr Asn Asp Val Cys Ser Gln Pro Leu Arg Gly His His Thr
 1605 1610 1615
 40 Thr Tyr Thr Glu Cys Cys Cys Gln Asp Gly Glu Ala Trp Ser Gln Gln
 1620 1625 1630
 Cys Ala Leu Cys Pro Pro Arg Ser Ser Glu Val Tyr Ala Gln Leu Cys
 1635 1640 1645
 45 Asn Val Ala Arg Ile Glu Ala Glu Arg Gly Ala Gly Ile His Phe Arg
 1650 1655 1660
 50 Pro Gly Tyr Glu Tyr Gly Pro Gly Leu Asp Asp Leu Pro Glu Asn Leu
 1665 1670 1675 1680
 Tyr Gly Pro Asp Gly Ala Pro Phe Tyr Asn Tyr Leu Gly Pro Glu Asp
 1685 1690 1695
 55 Thr Ala Pro Glu Pro Pro Phe Ser Asn Pro Ala Ser Gln Pro Gly Asp
 1700 1705 1710
 Asn Thr Pro Val Leu Glu Pro Pro Leu Gln Pro Ser Glu Leu Gln Pro
 1715 1720 1725
 60 His Tyr Leu Ala Ser His Ser Glu Pro Pro Ala Ser Phe Glu Gly Leu
 1730 1735 1740

Gln Ala Glu Glu Cys Gly Ile Leu Asn Gly Cys Glu Asn Gly Arg Cys
 1745 1750 1755 1760

Val Arg Val Arg Glu Gly Tyr Thr Cys Asp Cys Phe Glu Gly Phe Gln
 1765 1770 1775

Leu Asp Ala Pro Thr Leu Ala Cys Val Asp Val Asn Glu Cys Glu Asp
 1780 1785 1790

Leu Asn Gly Pro Ala Arg Leu Cys Ala His Gly His Cys Glu Asn Thr
 1795 1800 1805

Glu Gly Ser Tyr Arg Cys His Cys Ser Pro Gly Tyr Val Ala Glu Pro
 1810 1815 1820

Gly Pro Pro His Cys Ala Ala Lys Glu
 1825 1830

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3753 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "DNA"
- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..3753
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG CGC CAG GCC GCA TTG GGG CTG CTG GCA CTA CTC CTG CTG GCG CTG	48
Met Arg Gln Ala Ala Leu Gly Leu Leu Ala Leu Leu Leu Ala Leu	
1 5 10 15	
CTG GGC CCC GGC GGC CGA GGG GTG GGC CGG CCG GGC AGC GGG GCA CAG	96
Leu Gly Pro Gly Arg Gly Val Gly Arg Pro Gly Ser Gly Ala Gln	
20 25 30	
GCG GGG GCG GGG CGC TGG GCC CAA CGC TTC AAG GTG GTC TTT GCG CCT	144
Ala Gly Ala Gly Arg Trp Ala Gln Arg Phe Lys Val Val Phe Ala Pro	
35 40 45	
GTG ATC TGC AAG CGG ACC TGT CTG AAG GGC CAG TGT CGG GAC AGC TGT	192
Val Ile Cys Lys Arg Thr Cys Leu Lys Gly Gln Cys Arg Asp Ser Cys	
50 55 60	
CAG CAG GGC TCC AAC ATG ACG CTC ATC GGA GAG AAC GGC CAC AGC ACC	240
Gln Gln Gly Ser Asn Met Thr Leu Ile Gly Glu Asn Gly His Ser Thr	
65 70 75 80	
GAC ACG CTC ACC GGT TCT GCC TTC CGC GTG GTG GTG TGC CCT CTA CCC	288
Asp Thr Leu Thr Gly Ser Ala Phe Arg Val Val Val Cys Pro Leu Pro	
85 90 95	
TGC ATG AAC GGT GGC CAG TGC TCT TCC CGA AAC CAG TGC CTG TGT CCC	336
Cys Met Asn Gly Gly Gln Cys Ser Ser Arg Asn Gln Cys Leu Cys Pro	

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	CCT	GGC	TCT	TAT	CGC	TGT	GTC	TGC	CCG	CCC	GGT	CAT	AGC	TTG	GGT	CCC	1104
	Pro	Gly	Ser	Tyr	Arg	Cys	Val	Cys	Pro	Pro	Gly	His	Ser	Leu	Gly	Pro	
			355					360					365				
5	CTC	GCA	GCA	CAG	TGC	ATT	GCC	GAC	AAA	CCA	GAG	GAG	AAG	AGC	CTG	TGT	1152
	Leu	Ala	Ala	Gln	Cys	Ile	Ala	Asp	Lys	Pro	Glu	Glu	Lys	Ser	Leu	Cys	
		370					375					380					
10	TTC	CGC	CTT	GTG	AGC	ACC	GAA	CAC	CAG	TGC	CAG	CAC	CCT	CTG	ACC	ACA	1200
	Phe	Arg	Leu	Val	Ser	Thr	Glu	His	Gln	Cys	Gln	His	Pro	Leu	Thr	Thr	
		385					390				395					400	
15	CGC	CTA	ACC	CGC	CAG	CTC	TGC	TGC	TGT	AGT	GTG	GGT	AAA	GCC	TGG	GGT	1248
	Arg	Leu	Thr	Arg	Gln	Leu	Cys	Cys	Cys	Ser	Val	Gly	Lys	Ala	Trp	Gly	
					405					410					415		
20	GCC	CGG	TGC	CAG	CGC	TGC	CCG	GCA	GAT	GGT	ACA	GCA	GCC	TTC	AAG	GAG	1296
	Ala	Arg	Cys	Gln	Arg	Cys	Pro	Ala	Asp	Gly	Thr	Ala	Ala	Phe	Lys	Glu	
				420					425					430			
25	ATC	TGC	CCC	GGC	TGG	GAA	AGG	GTA	CCA	TAT	CCT	CAC	CTC	CCA	CCA	GAC	1344
	Ile	Cys	Pro	Gly	Trp	Glu	Arg	Val	Pro	Tyr	Pro	His	Leu	Pro	Pro	Asp	
			435					440					445				
30	GCT	CAC	CAT	CCA	GGG	GGA	AAG	CGA	CTT	CTC	CCT	CTT	CCT	GCA	CCC	GAC	1392
	Ala	His	His	Pro	Gly	Gly	Lys	Arg	Leu	Leu	Pro	Leu	Pro	Ala	Pro	Asp	
		450					455					460					
35	GGG	CCA	CCC	AAA	CCC	CAG	CAG	CTT	CCT	GAA	AGC	CCC	AGC	CGA	GCA	CCA	1440
	Gly	Pro	Pro	Lys	Pro	Gln	Gln	Leu	Pro	Glu	Ser	Pro	Ser	Arg	Ala	Pro	
		465				470					475					480	
40	CCC	CTC	GAG	GAC	ACA	GAG	GAA	GAG	AGA	GGA	GTG	ACC	ATG	GAT	CCA	CCA	1488
	Pro	Leu	Glu	Asp	Thr	Glu	Glu	Glu	Arg	Gly	Val	Thr	Met	Asp	Pro	Pro	
					485					490					495		
45	GTG	AGT	GAG	GAG	CGA	TCG	GTG	CAG	CAG	AGC	CAC	CCC	ACT	ACC	ACC	ACC	1536
	Val	Ser	Glu	Glu	Arg	Ser	Val	Gln	Gln	Ser	His	Pro	Thr	Thr	Thr	Thr	
					500				505					510			
50	TCA	CCC	CCC	CGG	CCT	TAC	CCA	GAG	CTC	ATC	TCT	CGC	CCC	TCC	CCA	CCT	1584
	Ser	Pro	Pro	Arg	Pro	Tyr	Pro	Glu	Leu	Ile	Ser	Arg	Pro	Ser	Pro	Pro	
			515					520					525				
55	ACC	TTC	CAC	CGG	TTC	CTG	CCA	GAC	TTG	CCC	CCA	TCC	CGA	AGT	GCA	GTG	1632
	Thr	Phe	His	Arg	Phe	Leu	Pro	Asp	Leu	Pro	Pro	Ser	Arg	Ser	Ala	Val	
		530					535					540					
60	GAG	ATC	GCC	CCC	ACT	CAG	GTC	ACA	GAG	ACC	GAT	GAG	TGC	CGA	TTG	AAC	1680
	Glu	Ile	Ala	Pro	Thr	Gln	Val	Thr	Glu	Thr	Asp	Glu	Cys	Arg	Leu	Asn	
		545				550					555					560	
65	CAG	AAT	ATC	TGT	GGC	CAT	GGA	CAG	TGT	GTG	CCT	GGC	CCC	TCG	GAT	TAC	1728
	Gln	Asn	Ile	Cys	Gly	His	Gly	Gln	Cys	Val	Pro	Gly	Pro	Ser	Asp	Tyr	
					565					570					575		
70	TCC	TGC	CAC	TGC	AAC	GCT	GGC	TAC	CGG	TCA	CAC	CCG	CAG	CAC	CGC	TAC	1776
	Ser	Cys	His	Cys	Asn	Ala	Gly	Tyr	Arg	Ser	His	Pro	Gln	His	Arg	Tyr	
					580				585					590			
75	TGT	GTT	GAT	GTG	AAC	GAG	TGC	GAG	GCA	GAG	CCC	TGC	GGC	CCC	GGG	AAA	1824
	Cys	Val	Asp	Val	Asn	Glu	Cys	Glu	Ala	Glu	Pro	Cys	Gly	Pro	Gly	Lys	

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	595						600						605						
5	GGC Gly 610	ATC Ile	TGT Cys	ATG Met	AAC Asn	ACT Thr	GGT Gly 615	GGC Gly	TCC Ser	TAC Tyr	AAT Asn	TGT Cys 620	CAC His	TGC Cys	AAC Asn	CGA Arg	1872		
10	GGC Gly 625	TAC Tyr	CGC Arg	CTC Leu	CAC His	GTG Val 630	GGT Gly 635	GCA Ala	GGG Gly	GGC Gly	CGC Arg	TCG Ser	TGC Cys	GTG Val	GAC Asp	CTG Leu 640	1920		
15	AAC Asn	GAG Glu	TGC Cys	GCC Ala	AAG Lys 645	CCT Pro	CAC His	CTG Leu	TGT Cys	GGG Gly 650	GAC Asp	GGT Gly	GGC Gly	TTC Phe	TGC Cys 655	ATC Ile	1968		
20	AAC Asn	TTC Phe	CCT Pro	GGT Gly 660	CAC His	TAC Tyr	AAA Lys	TGC Cys 665	AAC Asn	TGC Cys	TAT Tyr	CCT Pro	GGC Gly	TAC Tyr 670	CGG Arg	CTC Leu	2016		
25	AAG Lys	GCC Ala	TCC Ser 675	CGA Arg	CCG Pro	CCC Pro	ATT Ile	TGC Cys 680	GAA Glu	GAC Asp	ATC Ile	GAC Asp	GAG Glu 685	TGT Cys	CGC Arg	GAC Asp	2064		
30	CCT Pro	AGC Ser 690	ACC Thr	TGC Cys	CCT Pro	GAT Asp	GGC Gly 695	AAA Lys	TGT Cys	GAA Glu	AAC Asn	AAA Lys 700	CCT Pro	GGC Gly	AGC Ser	TTC Phe	2112		
35	AAG Lys 705	TGC Cys	ATC Ile	GCC Ala	TGC Cys	CAG Gln 710	CCT Pro	GGC Gly	TAC Tyr	CGT Arg	AGC Ser 715	CAG Gln	GGG Gly	GGC Gly	GGG Gly	GCC Ala 720	2160		
40	TGT Cys	CGT Arg	GAT Asp	GTC Val	AAC Asn 725	GAA Glu	TGC Cys	TCC Ser	GAA Glu	GGT Gly 730	ACC Thr	CCC Pro	TGC Cys	TCT Ser	CCT Pro 735	GGA Gly	2208		
45	TGG Trp	TGT Cys	GAG Glu 740	AAA Lys	CTT Leu	CCG Pro	GGT Gly	TCT Ser 745	TAC Tyr	CGT Arg	TGC Cys	ACG Thr	TGT Cys	GCC Ala 750	CAG Gln	GGG Gly	2256		
50	ATA Ile	CGA Arg	ACC Thr 755	CGC Arg	ACA Thr	GGA Gly	CGC Arg	CTC Leu 760	AGT Ser	TGC Cys	ATA Ile	GAC Asp	GTG Val 765	GAT Asp	GAC Asp	TGT Cys	2304		
55	GAG Glu 770	GCT Ala	GGG Gly	AAA Lys	GTG Val	TGC Cys	CAA Gln 775	GAT Asp	GGC Gly	ATC Ile	TGC Cys	ACG Thr 780	AAC Asn	ACA Thr	CCA Pro	GGC Gly	2352		
60	TCT Ser 785	TTC Phe	CAG Gln	TGT Cys	CAG Gln 790	TGC Cys	CTC Leu	TCC Ser	GGC Gly	TAT Tyr 795	CAT His	CTG Leu	TCA Ser	AGG Arg	GAT Asp 800	CGG Arg	2400		
65	AGC Ser	CGC Arg	TGT Cys	GAG Glu	GAC Asp 805	ATT Ile	GAT Asp	GAA Glu	TGT Cys	GAC Asp 810	TTC Phe	CCT Pro	GCG Ala	GCC Ala	TGC Cys 815	ATC Ile	2448		
70	GGG Gly	GGT Gly	GAC Asp	TGC Cys	ATC Ile	AAT Asn	ACC Thr	AAT Asn 825	GGT Gly	TCC Ser	TAC Tyr	AGA Arg	TGT Cys	CTC Leu 830	TGT Cys	CCC Pro	2496		
75	CTG Leu	GGT Gly	CAT His 835	CGG Arg	TTG Leu	GTG Val	GGC Gly 840	GGC Gly	AGG Arg	AAG Lys	TGC Cys	AAG Lys	AAA Lys 845	GAT Asp	ATA Ile	GAT Asp	2544		

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		GAG	TGC	AGC	CAG	GAC	CCA	GGC	CTG	TGC	CTG	CCC	CAT	GCC	TGC	GAG	AAC	2592
		Glu	Cys	Ser	Gln	Asp	Pro	Gly	Leu	Cys	Leu	Pro	His	Ala	Cys	Glu	Asn	
		850						855					860					
5		CTC	CAG	GGC	TCC	TAT	GTC	TGT	GTC	TGT	GAT	GAG	GGT	TTC	ACA	CTC	ACC	2640
		Leu	Gln	Gly	Ser	Tyr	Val	Cys	Val	Cys	Asp	Glu	Gly	Phe	Thr	Leu	Thr	
		865					870					875					880	
10		CAG	GAC	CAG	CAT	GGG	TGT	GAG	GAG	GTG	GAG	CAG	CCC	CAC	CAC	AAG	AAG	2688
		Gln	Asp	Gln	His	Gly	Cys	Glu	Glu	Val	Glu	Gln	Pro	His	His	Lys	Lys	
						885						890					895	
15		GAG	TGC	TAC	CTT	AAC	TTC	GAT	GAC	ACA	GTG	TTC	TGT	GAC	AGC	GTA	TTG	2736
		Glu	Cys	Tyr	Leu	Asn	Phe	Asp	Asp	Thr	Val	Phe	Cys	Asp	Ser	Val	Leu	
					900						905					910		
20		GCT	ACC	AAT	GTC	ACT	CAG	CAG	GAA	TGC	TGT	TGC	TCT	CTG	GGA	GCT	GGC	2784
		Ala	Thr	Asn	Val	Thr	Gln	Gln	Glu	Cys	Cys	Cys	Ser	Leu	Gly	Ala	Gly	
					915					920					925			
		TGG	GGA	GAC	CAC	TGC	GAA	ATC	TAT	CCC	TGT	CCA	GTC	TAC	AGC	TCA	GCC	2832
		Trp	Gly	Asp	His	Cys	Glu	Ile	Tyr	Pro	Cys	Pro	Val	Tyr	Ser	Ser	Ala	
			930					935						940				
25		GAA	TTT	CAC	AGC	CTG	GTG	CCT	GAT	GGG	AAA	AGG	CTA	CAC	TCA	GGA	CAA	2880
		Glu	Phe	His	Ser	Leu	Val	Pro	Asp	Gly	Lys	Arg	Leu	His	Ser	Gly	Gln	
		945					950					955					960	
30		CAA	CAT	TGT	GAA	CTA	TGC	ATT	CCT	GCC	CAC	CGT	GAC	ATC	GAC	GAA	TGC	2928
		Gln	His	Cys	Glu	Leu	Cys	Ile	Pro	Ala	His	Arg	Asp	Ile	Asp	Glu	Cys	
						965						970					975	
35		ATA	TTG	TTT	GGG	GCA	GAG	ATC	TGC	AAG	GAG	GGC	AAG	TGT	GTG	AAC	TCG	2976
		Ile	Leu	Phe	Gly	Ala	Glu	Ile	Cys	Lys	Glu	Gly	Lys	Cys	Val	Asn	Ser	
					980					985					990			
40		CAG	CCC	GGC	TAC	GAG	TGC	TAC	TGC	AAG	CAG	GGC	TTC	TAC	TAC	GAT	GGC	3024
		Gln	Pro	Gly	Tyr	Glu	Cys	Tyr	Cys	Lys	Gln	Gly	Phe	Tyr	Tyr	Asp	Gly	
				995					1000						1005			
45		AAC	CTG	CTG	GAG	TGC	GTG	GAC	GTG	GAC	GAG	TGC	TTG	GAT	GAG	TCT	AAC	3072
		Asn	Leu	Leu	Glu	Cys	Val	Asp	Val	Asp	Glu	Cys	Leu	Asp	Glu	Ser	Asn	
			1010					1015					1020					
50		TGC	AGG	AAC	GGA	GTG	TGT	GAG	AAC	ACG	TGG	CGG	CTA	CCG	TGT	GCC	TGC	3120
		Cys	Arg	Asn	Gly	Val	Cys	Glu	Asn	Thr	Trp	Arg	Leu	Pro	Cys	Ala	Cys	
		1025				1030						1035					1040	
55		ACT	CCG	CCG	GCA	GAG	TAC	AGT	CCC	GCA	CAG	GCC	CAG	TGT	CTG	ATC	CCG	3168
		Thr	Pro	Pro	Ala	Glu	Tyr	Ser	Pro	Ala	Gln	Ala	Gln	Cys	Leu	Ile	Pro	
						1045					1050					1055		
60		GAG	AGA	TGG	AGC	ACG	CCC	CAG	AGA	GAC	GTG	AAG	TGT	GCT	GGG	GCC	AGC	3216
		Glu	Arg	Trp	Ser	Thr	Pro	Gln	Arg	Asp	Val	Lys	Cys	Ala	Gly	Ala	Ser	
					1060					1065					1070			
		GAG	GAG	AGG	ACG	GCA	TGT	GTA	TGG	GGC	CCC	TGG	GCG	GGA	CCT	GCC	CTC	3264
		Glu	Glu	Arg	Thr	Ala	Cys	Val	Trp	Gly	Pro	Trp	Ala	Gly	Pro	Ala	Leu	
				1075					1080						1085			
60		ACT	TTT	GAT	GAC	TGC	TGC	TGC	CGC	CAG	CCG	CGG	CTG	GGT	ACC	CAG	TGC	3312
		Thr	Phe	Asp	Asp	Cys	Cys	Cys	Arg	Gln	Pro	Arg	Leu	Gly	Thr	Gln	Cys	

1090

1095

1100

AGA CCG TGC CCG CCA CGT GGC ACC GGG TCC CAG TGC CCG ACT TCA CAG 3360
 Arg Pro Cys Pro Pro Arg Gly Thr Gly Ser Gln Cys Pro Thr Ser Gln
 1105 1110 1115 1120

AGT GAG AGC AAT TCT TTC TGG GAC ACA AGC CCC CTG CTA CTG GGG AAG 3408
 Ser Glu Ser Asn Ser Phe Trp Asp Thr Ser Pro Leu Leu Leu Gly Lys
 1125 1130 1135

TCT CCG CGA GAC GAA GAC AGC TCA GAG GAG GAT TCA GAT GAG TGC CGT 3456
 Ser Pro Arg Asp Glu Asp Ser Ser Glu Glu Asp Ser Asp Glu Cys Arg
 1140 1145 1150

TGT GTG AGC GGA CCG TGT GTG CCA CGG CCA GGC GGG GCG GTA TGC GAG 3504
 Cys Val Ser Gly Pro Cys Val Pro Arg Pro Gly Gly Ala Val Cys Glu
 1155 1160 1165

TGT CCT GGA GGC TTT CAG CTG GAC GCC TCC CGT GCC CGC TGC GTG GAC 3552
 Cys Pro Gly Gly Phe Gln Leu Asp Ala Ser Arg Ala Arg Cys Val Asp
 1170 1175 1180

ATT GAT GAG TGC CGA GAA CTG AAC CAG CGG GGA CTG CTG TGT AAG AGC 3600
 Ile Asp Glu Cys Arg Glu Leu Asn Gln Arg Gly Leu Leu Cys Lys Ser
 1185 1190 1195 1200

GAG CGG TGC GTG AAC ACC AGT GGA TCC TTC CGC TGT GTC TGC AAA GCT 3648
 Glu Arg Cys Val Asn Thr Ser Gly Ser Phe Arg Cys Val Cys Lys Ala
 1205 1210 1215

GGC TTC ACG CGC AGC CGC CCT CAC GGG CCT GCG TGC CTC AGC GCC GCC 3696
 Gly Phe Thr Arg Ser Arg Pro His Gly Pro Ala Cys Leu Ser Ala Ala
 1220 1225 1230

GCT GAT GAT GCA GCC ATA GCC CAC ACC TCA GTG ATC GAT CAT CGA GGG 3744
 Ala Asp Asp Ala Ala Ile Ala His Thr Ser Val Ile Asp His Arg Gly
 1235 1240 1245

TAT TTT CAC 3753
 Tyr Phe His
 1250

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1251 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Arg Gln Ala Ala Leu Gly Leu Leu Ala Leu Leu Leu Leu Ala Leu
 1 5 10 15
 Leu Gly Pro Gly Gly Arg Gly Val Gly Arg Pro Gly Ser Gly Ala Gln
 20 25 30
 Ala Gly Ala Gly Arg Trp Ala Gln Arg Phe Lys Val Val Phe Ala Pro
 35 40 45

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	Val	Ile	Cys	Lys	Arg	Thr	Cys	Leu	Lys	Gly	Gln	Cys	Arg	Asp	Ser	Cys	
	50						55					60					
5	Gln	Gln	Gly	Ser	Asn	Met	Thr	Leu	Ile	Gly	Glu	Asn	Gly	His	Ser	Thr	
	65					70					75					80	
	Asp	Thr	Leu	Thr	Gly	Ser	Ala	Phe	Arg	Val	Val	Val	Cys	Pro	Leu	Pro	
					85					90					95		
10	Cys	Met	Asn	Gly	Gly	Gln	Cys	Ser	Ser	Arg	Asn	Gln	Cys	Leu	Cys	Pro	
				100					105					110			
	Pro	Asp	Phe	Thr	Gly	Arg	Phe	Cys	Gln	Val	Pro	Ala	Ala	Gly	Thr	Gly	
			115					120					125				
15	Ala	Gly	Thr	Gly	Ser	Ser	Gly	Pro	Gly	Trp	Pro	Asp	Arg	Ala	Met	Ser	
		130					135					140					
	Thr	Gly	Pro	Leu	Pro	Pro	Leu	Ala	Pro	Glu	Gly	Glu	Ser	Val	Ala	Ser	
20		145				150					155					160	
	Lys	His	Ala	Ile	Tyr	Ala	Val	Gln	Val	Ile	Ala	Asp	Pro	Pro	Gly	Pro	
				165						170					175		
25	Gly	Glu	Gly	Pro	Pro	Ala	Gln	His	Ala	Ala	Phe	Leu	Val	Pro	Leu	Gly	
				180					185					190			
	Pro	Gly	Gln	Ile	Ser	Ala	Glu	Val	Gln	Ala	Pro	Pro	Pro	Val	Val	Asn	
			195					200					205				
30	Val	Arg	Val	His	His	Pro	Pro	Glu	Ala	Ser	Val	Gln	Val	His	Arg	Ile	
		210					215					220					
	Glu	Gly	Pro	Asn	Ala	Glu	Gly	Pro	Ala	Ser	Ser	Gln	His	Leu	Leu	Pro	
	225					230					235					240	
35	His	Pro	Lys	Pro	Pro	His	Pro	Arg	Pro	Pro	Thr	Gln	Lys	Pro	Leu	Gly	
					245					250					255		
40	Arg	Cys	Phe	Gln	Asp	Thr	Leu	Pro	Lys	Gln	Pro	Cys	Gly	Ser	Asn	Pro	
				260					265					270			
	Leu	Pro	Gly	Leu	Thr	Lys	Gln	Glu	Asp	Cys	Cys	Gly	Ser	Ile	Gly	Thr	
			275					280					285				
45	Ala	Trp	Gly	Gln	Ser	Lys	Cys	His	Lys	Cys	Pro	Gln	Leu	Gln	Tyr	Thr	
		290					295					300					
	Gly	Val	Gln	Lys	Pro	Val	Pro	Val	Arg	Gly	Glu	Val	Gly	Ala	Asp	Cys	
50		305				310					315					320	
	Pro	Gln	Gly	Tyr	Lys	Arg	Leu	Asn	Ser	Thr	His	Cys	Gln	Asp	Ile	Asn	
				325						330					335		
55	Glu	Cys	Ala	Met	Pro	Gly	Asn	Val	Cys	His	Gly	Asp	Cys	Leu	Asn	Asn	
				340					345					350			
	Pro	Gly	Ser	Tyr	Arg	Cys	Val	Cys	Pro	Pro	Gly	His	Ser	Leu	Gly	Pro	
			355					360					365				
60	Leu	Ala	Ala	Gln	Cys	Ile	Ala	Asp	Lys	Pro	Glu	Glu	Lys	Ser	Leu	Cys	
		370					375					380					

005599.0620045

	Phe	Arg	Leu	Val	Ser	Thr	Glu	His	Gln	Cys	Gln	His	Pro	Leu	Thr	Thr	385	390	395	400
5	Arg	Leu	Thr	Arg	Gln	Leu	Cys	Cys	Cys	Ser	Val	Gly	Lys	Ala	Trp	Gly	405	410	415	
	Ala	Arg	Cys	Gln	Arg	Cys	Pro	Ala	Asp	Gly	Thr	Ala	Ala	Phe	Lys	Glu	420	425	430	
10	Ile	Cys	Pro	Gly	Trp	Glu	Arg	Val	Pro	Tyr	Pro	His	Leu	Pro	Pro	Asp	435	440	445	
	Ala	His	His	Pro	Gly	Gly	Lys	Arg	Leu	Leu	Pro	Leu	Pro	Ala	Pro	Asp	450	455	460	
15	Gly	Pro	Pro	Lys	Pro	Gln	Gln	Leu	Pro	Glu	Ser	Pro	Ser	Arg	Ala	Pro	465	470	475	480
	Pro	Leu	Glu	Asp	Thr	Glu	Glu	Glu	Arg	Gly	Val	Thr	Met	Asp	Pro	Pro	485	490	495	
20	Val	Ser	Glu	Glu	Arg	Ser	Val	Gln	Gln	Ser	His	Pro	Thr	Thr	Thr	Thr	500	505	510	
	Ser	Pro	Pro	Arg	Pro	Tyr	Pro	Glu	Leu	Ile	Ser	Arg	Pro	Ser	Pro	Pro	515	520	525	
25	Thr	Phe	His	Arg	Phe	Leu	Pro	Asp	Leu	Pro	Pro	Ser	Arg	Ser	Ala	Val	530	535	540	
30	Glu	Ile	Ala	Pro	Thr	Gln	Val	Thr	Glu	Thr	Asp	Glu	Cys	Arg	Leu	Asn	545	550	555	560
	Gln	Asn	Ile	Cys	Gly	His	Gly	Gln	Cys	Val	Pro	Gly	Pro	Ser	Asp	Tyr	565	570	575	
35	Ser	Cys	His	Cys	Asn	Ala	Gly	Tyr	Arg	Ser	His	Pro	Gln	His	Arg	Tyr	580	585	590	
40	Cys	Val	Asp	Val	Asn	Glu	Cys	Glu	Ala	Glu	Pro	Cys	Gly	Pro	Gly	Lys	595	600	605	
	Gly	Ile	Cys	Met	Asn	Thr	Gly	Gly	Ser	Tyr	Asn	Cys	His	Cys	Asn	Arg	610	615	620	
45	Gly	Tyr	Arg	Leu	His	Val	Gly	Ala	Gly	Gly	Arg	Ser	Cys	Val	Asp	Leu	625	630	635	640
	Asn	Glu	Cys	Ala	Lys	Pro	His	Leu	Cys	Gly	Asp	Gly	Gly	Phe	Cys	Ile	645	650	655	
50	Asn	Phe	Pro	Gly	His	Tyr	Lys	Cys	Asn	Cys	Tyr	Pro	Gly	Tyr	Arg	Leu	660	665	670	
55	Lys	Ala	Ser	Arg	Pro	Pro	Ile	Cys	Glu	Asp	Ile	Asp	Glu	Cys	Arg	Asp	675	680	685	
	Pro	Ser	Thr	Cys	Pro	Asp	Gly	Lys	Cys	Glu	Asn	Lys	Pro	Gly	Ser	Phe	690	695	700	
60	Lys	Cys	Ile	Ala	Cys	Gln	Pro	Gly	Tyr	Arg	Ser	Gln	Gly	Gly	Gly	Ala	705	710	715	720

	Cys	Arg	Asp	Val	Asn	Glu	Cys	Ser	Glu	Gly	Thr	Pro	Cys	Ser	Pro	Gly
					725					730						735
5	Trp	Cys	Glu	Lys	Leu	Pro	Gly	Ser	Tyr	Arg	Cys	Thr	Cys	Ala	Gln	Gly
				740					745					750		
	Ile	Arg	Thr	Arg	Thr	Gly	Arg	Leu	Ser	Cys	Ile	Asp	Val	Asp	Asp	Cys
			755					760					765			
10	Glu	Ala	Gly	Lys	Val	Cys	Gln	Asp	Gly	Ile	Cys	Thr	Asn	Thr	Pro	Gly
		770					775					780				
	Ser	Phe	Gln	Cys	Gln	Cys	Leu	Ser	Gly	Tyr	His	Leu	Ser	Arg	Asp	Arg
	785					790					795					800
15	Ser	Arg	Cys	Glu	Asp	Ile	Asp	Glu	Cys	Asp	Phe	Pro	Ala	Ala	Cys	Ile
					805					810					815	
20	Gly	Gly	Asp	Cys	Ile	Asn	Thr	Asn	Gly	Ser	Tyr	Arg	Cys	Leu	Cys	Pro
				820					825					830		
	Leu	Gly	His	Arg	Leu	Val	Gly	Gly	Arg	Lys	Cys	Lys	Lys	Asp	Ile	Asp
			835					840					845			
25	Glu	Cys	Ser	Gln	Asp	Pro	Gly	Leu	Cys	Leu	Pro	His	Ala	Cys	Glu	Asn
		850					855					860				
	Leu	Gln	Gly	Ser	Tyr	Val	Cys	Val	Cys	Asp	Glu	Gly	Phe	Thr	Leu	Thr
	865					870					875					880
30	Gln	Asp	Gln	His	Gly	Cys	Glu	Glu	Val	Glu	Gln	Pro	His	His	Lys	Lys
					885					890					895	
35	Glu	Cys	Tyr	Leu	Asn	Phe	Asp	Asp	Thr	Val	Phe	Cys	Asp	Ser	Val	Leu
				900					905					910		
	Ala	Thr	Asn	Val	Thr	Gln	Gln	Glu	Cys	Cys	Cys	Ser	Leu	Gly	Ala	Gly
			915					920					925			
40	Trp	Gly	Asp	His	Cys	Glu	Ile	Tyr	Pro	Cys	Pro	Val	Tyr	Ser	Ser	Ala
		930					935					940				
	Glu	Phe	His	Ser	Leu	Val	Pro	Asp	Gly	Lys	Arg	Leu	His	Ser	Gly	Gln
	945					950					955					960
45	Gln	His	Cys	Glu	Leu	Cys	Ile	Pro	Ala	His	Arg	Asp	Ile	Asp	Glu	Cys
					965					970					975	
50	Ile	Leu	Phe	Gly	Ala	Glu	Ile	Cys	Lys	Glu	Gly	Lys	Cys	Val	Asn	Ser
				980					985					990		
	Gln	Pro	Gly	Tyr	Glu	Cys	Tyr	Cys	Lys	Gln	Gly	Phe	Tyr	Tyr	Asp	Gly
			995					1000					1005			
55	Asn	Leu	Leu	Glu	Cys	Val	Asp	Val	Asp	Glu	Cys	Leu	Asp	Glu	Ser	Asn
		1010					1015					1020				
	Cys	Arg	Asn	Gly	Val	Cys	Glu	Asn	Thr	Trp	Arg	Leu	Pro	Cys	Ala	Cys
	1025					1030					1035					1040
60	Thr	Pro	Pro	Ala	Glu	Tyr	Ser	Pro	Ala	Gln	Ala	Gln	Cys	Leu	Ile	Pro

Glu Arg Trp Ser Thr Pro Gln Arg Asp Val Lys Cys Ala Gly Ala Ser
1060 1065 1070

Glu Glu Arg Thr Ala Cys Val Trp Gly Pro Trp Ala Gly Pro Ala Leu
1075 1080 1085

Thr Phe Asp Asp Cys Cys Cys Arg Gln Pro Arg Leu Gly Thr Gln Cys
1090 1095 1100

Arg Pro Cys Pro Pro Arg Gly Thr Gly Ser Gln Cys Pro Thr Ser Gln
1105 1110 1115 1120

Ser Glu Ser Asn Ser Phe Trp Asp Thr Ser Pro Leu Leu Leu Gly Lys
1125 1130 1135

Ser Pro Arg Asp Glu Asp Ser Ser Glu Glu Asp Ser Asp Glu Cys Arg
1140 1145 1150

Cys Val Ser Gly Pro Cys Val Pro Arg Pro Gly Gly Ala Val Cys Glu
1155 1160 1165

Cys Pro Gly Gly Phe Gln Leu Asp Ala Ser Arg Ala Arg Cys Val Asp
1170 1175 1180

Ile Asp Glu Cys Arg Glu Leu Asn Gln Arg Gly Leu Leu Cys Lys Ser
1185 1190 1195 1200

Glu Arg Cys Val Asn Thr Ser Gly Ser Phe Arg Cys Val Cys Lys Ala
1205 1210 1215

Gly Phe Thr Arg Ser Arg Pro His Gly Pro Ala Cys Leu Ser Ala Ala
1220 1225 1230

Ala Asp Asp Ala Ala Ile Ala His Thr Ser Val Ile Asp His Arg Gly
1235 1240 1245

Tyr Phe His
1250

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gly Glu Ser Val Ala Ser Lys His Ala Ile Tyr Ala Val Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
 TACCGATGCT ACCGCAGCAA TCTT 24

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
 ATGCCTAAAC TCTACCAGCA CG 22

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
 GAGTCACGTC ATCCATTCCA CA 22

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
 CGTCCAAGTT GTGTCTTAGC AG 22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 53 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gly Pro Pro Gly Pro Gln Gly Ala Thr Gly Pro Leu Gly Pro Lys Gly
1 5 10 15
Gln Thr Gly Glu Pro Gly Ile Ala Gly Phe Lys Gly Glu Gln Gly Pro
20 25 30
Lys Gly Glu Thr Gly Pro Ala Gly Pro Gln Gly Ala Pro Gly Pro Ala
35 40 45
Gly Glu Glu Gly Lys
50

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 159 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGCCCTCCCG GTCCTCAAGG TGCAACTGGT CCTCTGGGCC CCAAAGGTCA GACGGGTGAG 60
CCCGGCATCG CTGGCTTCAA AGGTGAACAA GGCCCCAAGG GAGAGACTGG ACCTGCTGGG 120
CCCCAGGGAG CCCCTGGCCC TGCTGGTGAA GAAGGAAAA 159

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AAACGTCACA CGTGANACGT GAACGTTGCT TGCTGG 35

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- 5 (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA"
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

10 TTACGTCCAC GTACACGTCT AGCAAGCAAG CA

32

WHAT IS CLAIMED IS:

1. A DNA segment comprising an isolated gene that encodes an LTBP-2 or LTBP-3 protein.
2. The DNA segment of claim 1, comprising an isolated gene that encodes an LTBP-2 protein.
3. The DNA segment of claim 2, comprising an LTBP-2 gene that encodes an LTBP-2 protein or peptide that includes a contiguous amino acid sequence from SEQ ID NO:2.
4. The DNA segment of claim 3, comprising an LTBP-2 gene that includes a contiguous nucleic acid sequence from between position 1 and position 5499 of SEQ ID NO:1.
5. The DNA segment of claim 3, comprising an LTBP-2 gene that encodes an LTBP-2 protein of about 1833 amino acids in length.
6. The DNA segment of claim 5, comprising an LTBP-2 gene that includes the contiguous nucleic acid sequence from position 1 to position 5499 of SEQ ID NO:1.
7. The DNA segment of claim 1, comprising an isolated gene that encodes an LTBP-3 protein.

8. The DNA segment of claim 7, comprising an LTBP-3 gene that encodes an LTBP-3 protein or peptide that includes a contiguous amino acid sequence from SEQ ID NO:4.

9. The DNA segment of claim 8, comprising an LTBP-3 gene that includes a contiguous nucleic acid sequence from between position 1 and position 3753 of SEQ ID NO:3.

10. The DNA segment of claim 8, comprising an LTBP-3 gene that encodes an LTBP-3 protein of about 1251 amino acids in length.

11. The DNA segment of claim 10, comprising an LTBP-3 gene that includes the contiguous nucleic acid sequence from position 1 to position 3753 of SEQ ID NO:3.

12. The DNA segment of claim 1, positioned under the control of a promoter.

13. The DNA segment of claim 12, positioned under the control of a recombinant promoter.

14. The DNA segment of claim 12, wherein the isolated gene is positioned, in reverse orientation, under the control of a promoter that directs the expression of an antisense product.

15. The DNA segment of claim 13, further defined as a recombinant vector.

16. A recombinant host cell comprising a DNA segment that comprises an isolated gene that encodes an LTBP-2 or LTBP-3 protein or peptide.

5

17. The recombinant host cell of claim 15, further defined as a prokaryotic host cell.

18. The recombinant host cell of claim 15, further defined as a eukaryotic host cell.

10

19. The recombinant host cell of claim 15, wherein the DNA segment is introduced into the cell by means of a recombinant vector and the host cell expresses the DNA segment to produce the encoded LTBP-2 or LTBP-3 protein or peptide.

20. The recombinant host cell of claim 18, wherein the expressed LTBP-2 or LTBP-3 protein or peptide includes a contiguous amino acid sequence from SEQ ID NO:2 or SEQ ID NO:4.

21. A method of using a DNA segment that includes an isolated gene that encodes an LTBP-2 or LTBP-3 protein or peptide, comprising the steps of:

25

(a) preparing a recombinant vector in which an LTBP-2- or LTBP-3-encoding DNA segment is positioned under the control of a promoter;

(b) introducing said recombinant vector into a recombinant host cell;

30

(c) culturing the recombinant host cell under conditions effective to allow

expression of an encoded LTBP-2 or LTBP-3 protein or peptide; and

- (d) collecting said expressed LTBP-2 or LTBP-3 protein or peptide.

5

22. A recombinant LTBP-2 or LTBP-3 protein or peptide prepared by expressing an LTBP-2- or LTBP-3-encoding DNA segment in a recombinant host cell and purifying the expressed LTBP-2- or LTBP-3 protein or peptide away from total recombinant host cell components.

10

23. A method for detecting an LTBP-2 or LTBP-3 nucleic acid segment in a sample, comprising the steps of:

- (a) obtaining sample nucleic acids from a sample suspected of containing an LTBP-2 or LTBP-3 nucleic acid segment;

- (b) contacting said sample nucleic acids with an isolated LTBP-2 or LTBP-3 nucleic acid segment under conditions effective to allow hybridization of substantially complementary nucleic acids; and

- (c) detecting the hybridized complementary nucleic acids thus formed.

24. A nucleic acid segment characterized as:

- (a) a nucleic acid segment comprising a sequence region that consists of at least 17 contiguous nucleotides that have the same sequence as, or are complementary to, 17 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:3; or

30

- (b) a nucleic acid segment of from 17 to about 10,000 nucleotides in length that hybridizes to the nucleic acid segment of SEQ ID NO:1 or SEQ ID NO:3, or a complement thereof, under standard hybridization conditions.

5

25. The nucleic acid segment of claim 23, wherein the segment comprises a sequence region of at least 17 contiguous nucleotides from SEQ ID NO:1 or SEQ ID NO:3 or a complement thereof.

10

26. The nucleic acid segment of claim 23, wherein the segment hybridizes to the nucleic acid segment of SEQ ID NO:1 or SEQ ID NO:3 or a complement thereof.

27. The nucleic acid segment of claim 23, wherein the segment comprises a sequence region of at least 17 contiguous nucleotides from SEQ ID NO:1, or the complement thereof; or wherein the segment hybridizes to the nucleic acid segment of SEQ ID NO:1, or the complement thereof.

28. The nucleic acid segment of claim 23, wherein the segment comprises a sequence region of at least 17 contiguous nucleotides from SEQ ID NO:3, or the complement thereof; or wherein the segment hybridizes to the nucleic acid segment of SEQ ID NO:3, or the complement thereof.

29. The nucleic acid segment of claim 23, wherein the segment comprises a sequence region of at least about 25 nucleotides; or wherein the segment is about 25 nucleotides in length.

30. The nucleic acid segment of claim 28, wherein the segment comprises a sequence region of at least about 50 nucleotides; or wherein the segment is about 50 nucleotides in length.

5

31. The nucleic acid segment of claim 29, wherein the segment comprises a sequence region of at least about 100 nucleotides; or wherein the segment is about 100 nucleotides in length.

10

32. The nucleic acid segment of claim 30, wherein the segment comprises a sequence region that consists of the 5499 contiguous nucleotides of SEQ ID NO:1, or the complement thereof.

33. The nucleic acid segment of claim 30, wherein the segment comprises a sequence region that consists of the 3753 contiguous nucleotides of SEQ ID NO:3, or the complement thereof.

34. The nucleic acid segment of claim 23, wherein the segment is up to about 10,000 basepairs in length.

25

35. The nucleic acid segment of claim 33, wherein the segment is up to 5,000 basepairs in length.

30 36. The nucleic acid segment of claim 23, further defined as a RNA segment.

37. A purified antibody that binds to an LTBP-3 protein or peptide.

5

38. The antibody of claim 37, wherein the antibody is linked to a detectable label.

10 39. An immunodetection kit comprising, in suitable container means, an LTBP-3 protein or peptide, or a first antibody that binds to an LTBP-3 protein or peptide, and an immunodetection reagent.

15 40. A method for identifying a transforming growth factor β protein in a sample, comprising contacting said sample with an LTBP-2 or LTBP-3 protein under conditions effective to allow binding and detecting the protein so bound.

20 41. A method for purifying transforming growth factor β (TGF β) protein in a sample, comprising:

(a) contacting a sample suspected of containing TGF β β protein with an LTBP-2 or LTBP-3 protein under conditions effective to allow specific binding; and

(b) collecting the bound TGF β , substantially free from the non-bound components.

25

42. A composition comprising a purified murine LTBP-2 or LTBP-3 polypeptide.

ABSTRACT OF THE DISCLOSURE

Disclosed are novel nucleic acid and peptide compositions comprising latent TGF β binding proteins (LTBPs). Also disclosed are methods of using LTBP-2 and LTBP-3 peptides and the DNA segments which encode them.

5

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Carolanne M. King
Carolanne M. King



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Jeffery Bonadio
and Wushan Yin

Serial No.: Unknown

Filed: June 12, 2000

For: METHODS OF USING LATENT TGF β
BINDING PROTEIN-3 (LTBP-3)

Group Art Unit: 1646

Examiner: Fitzgerald, D.

Atty. Dkt. No.: 4100.000582

SUBMISSION OF FORMAL DRAWINGS

ATTN OFFICIAL DRAFTSMAN

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants hereby submit the formal drawings for the above-referenced application (FIGS. 1, 2A, 2B, 2C, 3A, 3B, 3C, 3D, 3E, 3F, 4, 5, 6, 7, 8A, 8B and 8C, making 8 different drawings on 8 sheets) and request that these drawings be accepted for filing.

002790 5826580

No fees are believed to be due in connection with the filing of these drawings, however, should any fees under 37 C.F.R. §§ 1.16 to 1.21 be deemed necessary for any reason relating to these materials, the Assistant Commissioner is hereby authorized to deduct said fees from Williams, Morgan & Amerson, P.C., Deposit Account No. 50-0786/4100.000582.

Respectfully submitted,



Shelley P.M. Fussey
Reg. No. 39,458
Agent for Applicants

WILLIAMS, MORGAN & AMERSON
7676 Hillmont, Suite 250
Houston, Texas 77040
(713) 934-7000

Date: June 12, 2000

002190"5892580

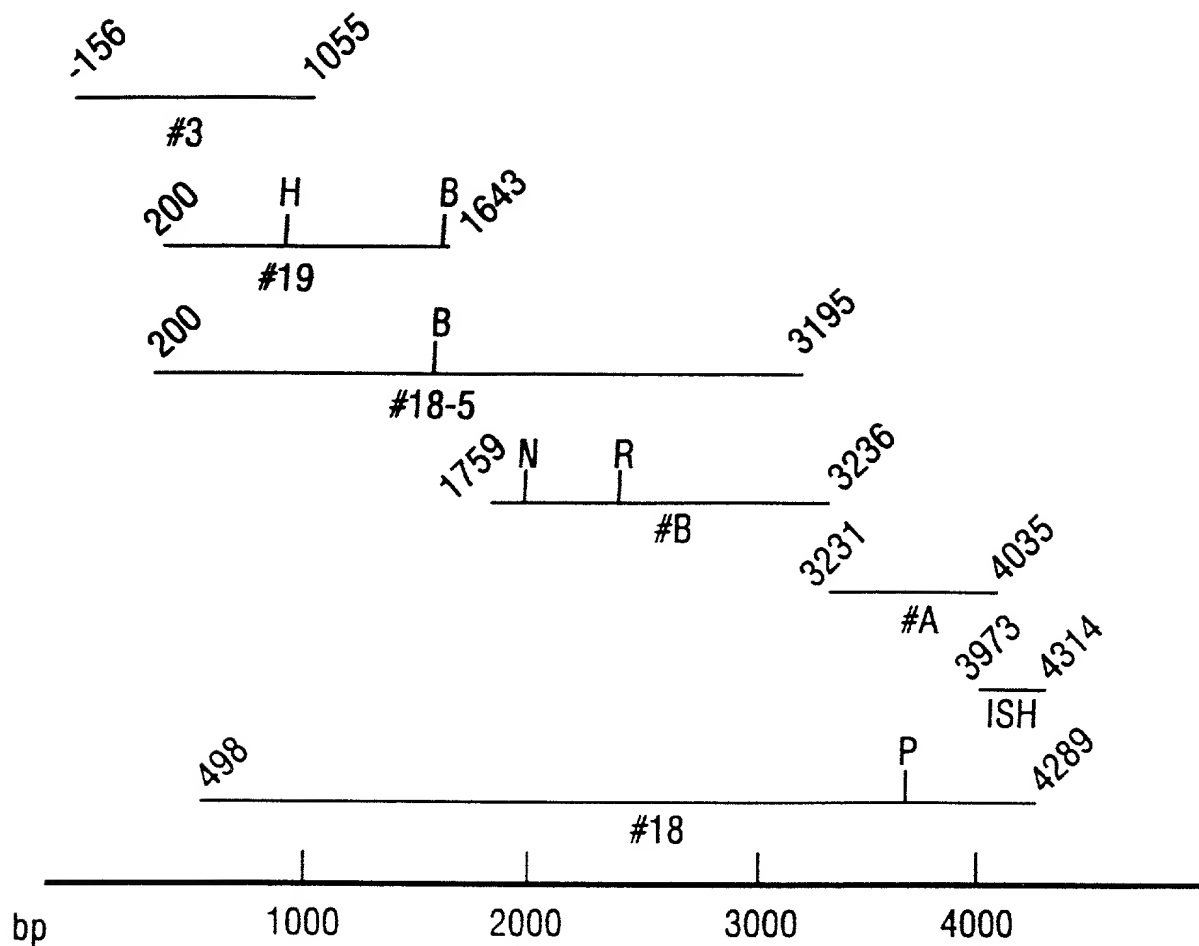


FIG. 1

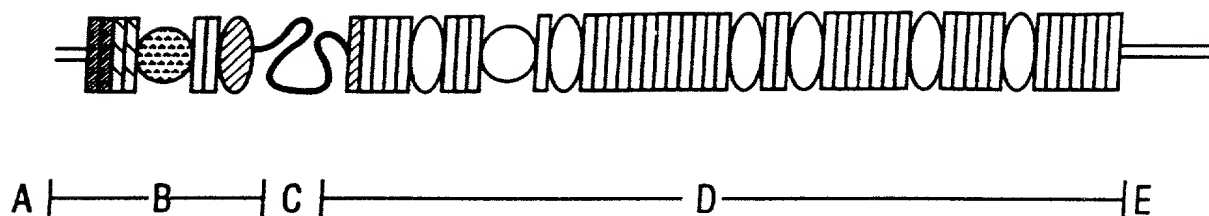


FIG. 2A

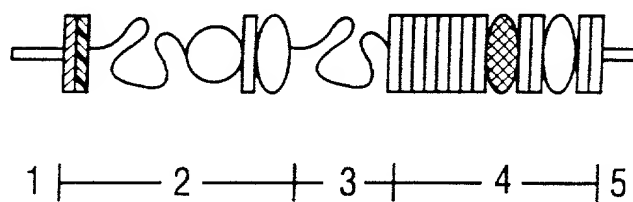


FIG. 2B

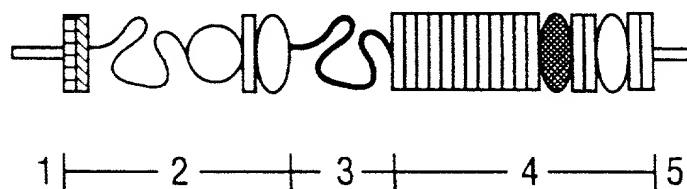


FIG. 2C

anti-sense
probe

FIG. 3A

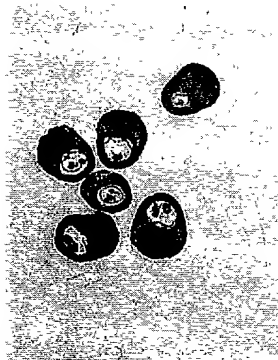


FIG. 3C

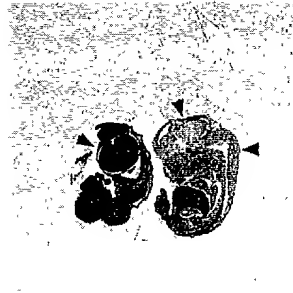


FIG. 3E



sense probe

day 8.5 - 9.0

FIG. 3B



day 13.5

FIG. 3D



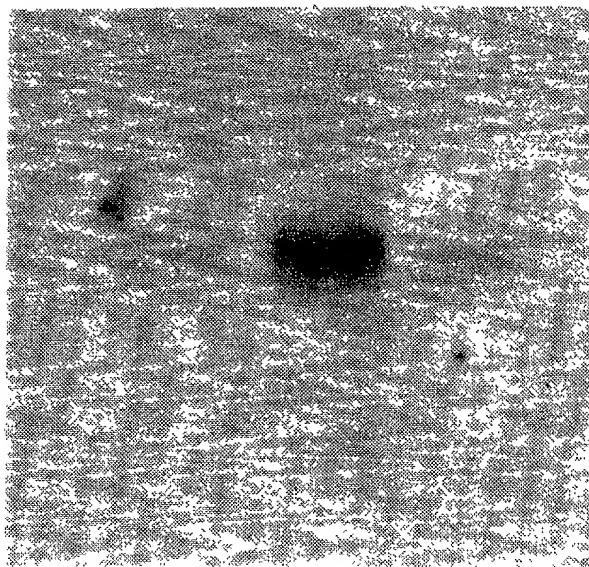
day 16.5

FIG. 3F



09592685-061200
002T90" 58926560

4.4 kb—



DAY

5

14

28

FIG. 4

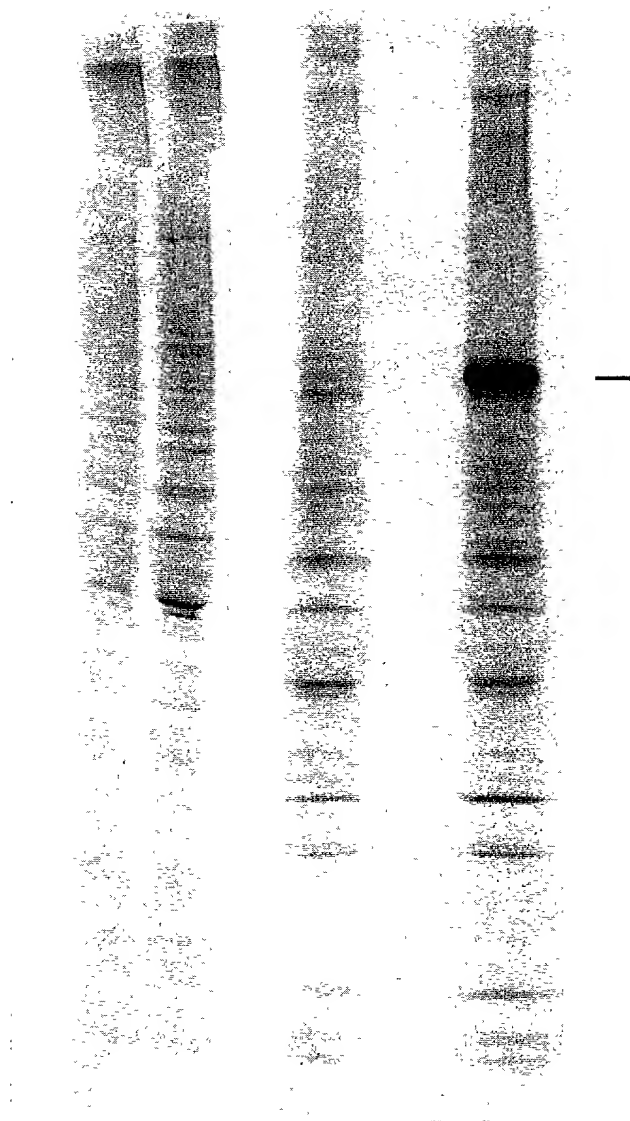


FIG. 5

002790" 58926560



FIG. 6

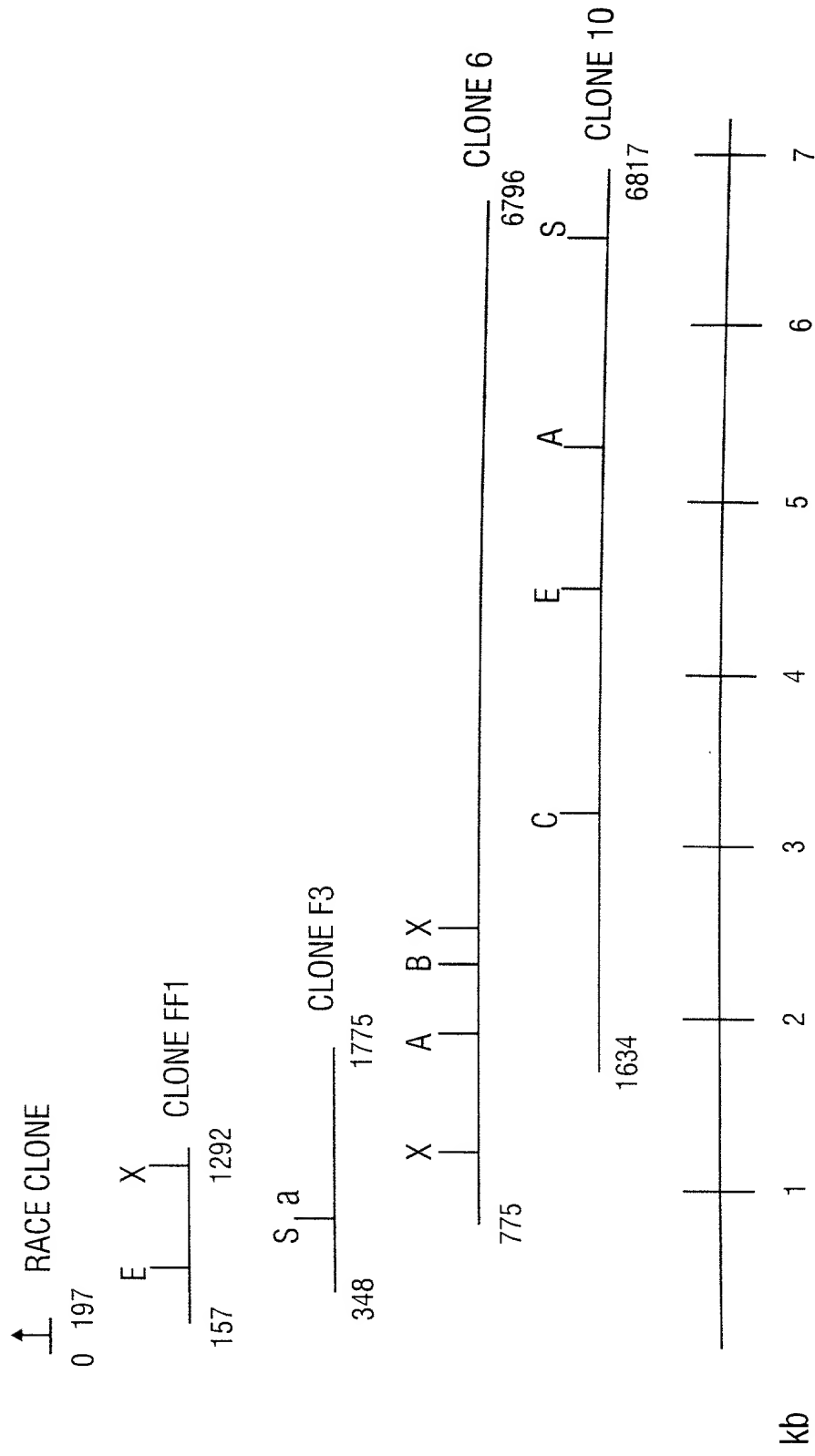


FIG. 7

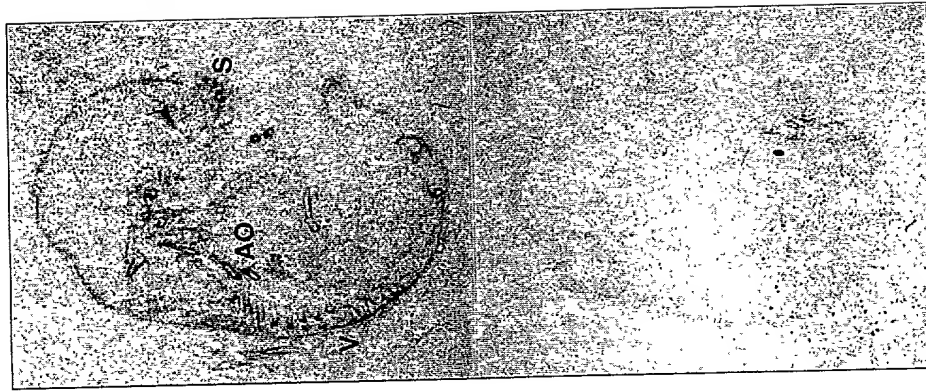


FIG. 8A

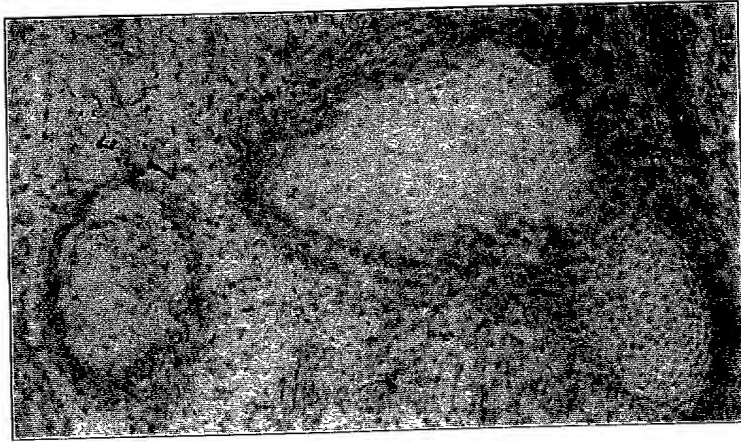


FIG. 8B



FIG. 8C

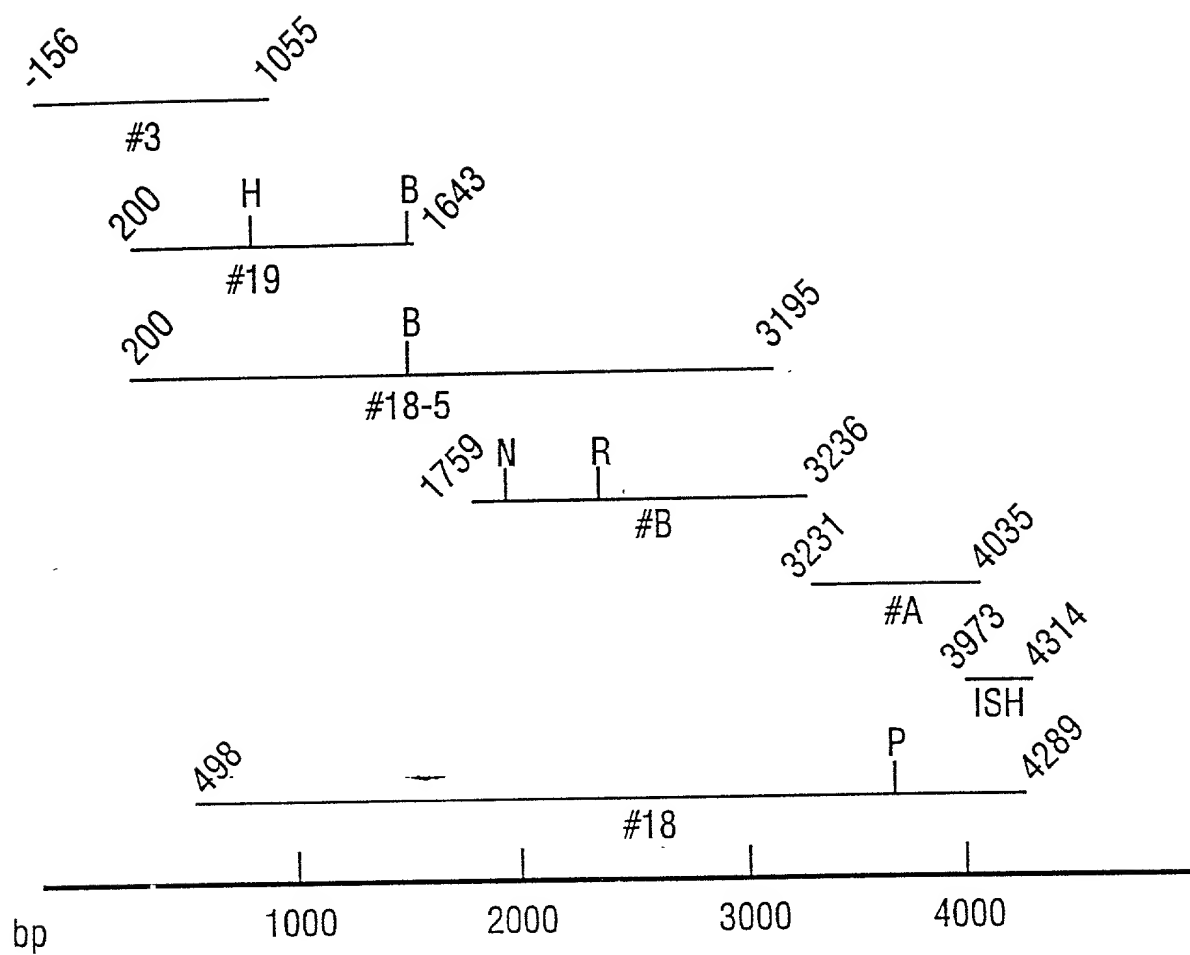


FIG. 1

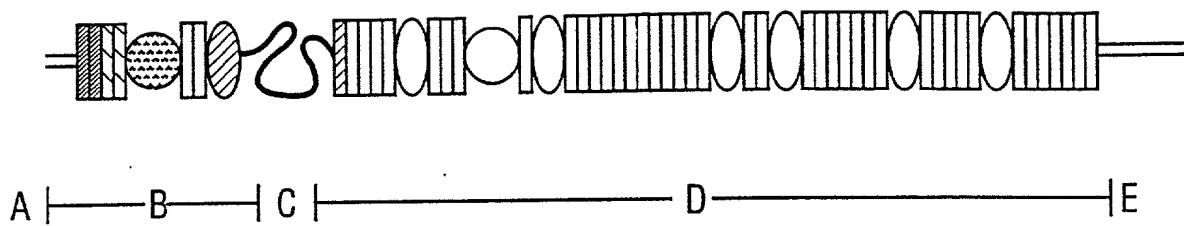


FIG. 2A

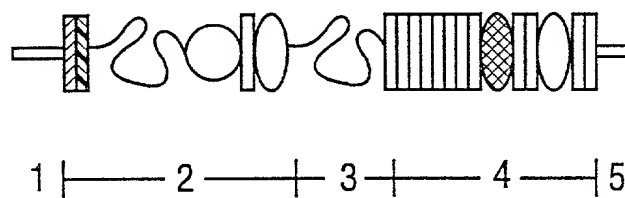


FIG. 2B

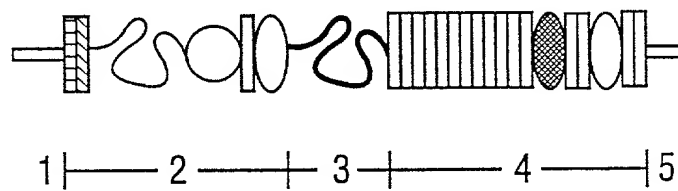


FIG. 2C

002790" 58926560

anti-sense probe

FIG. 3A

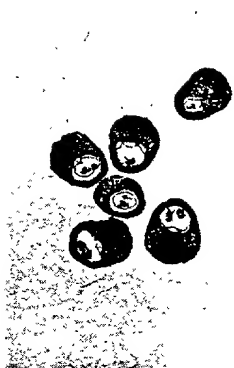


FIG. 3C



FIG. 3E



sense probe

FIG 3B



FIG. 3D

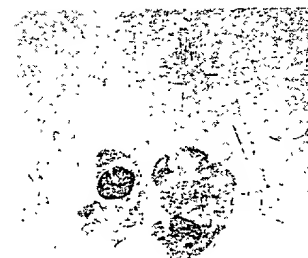
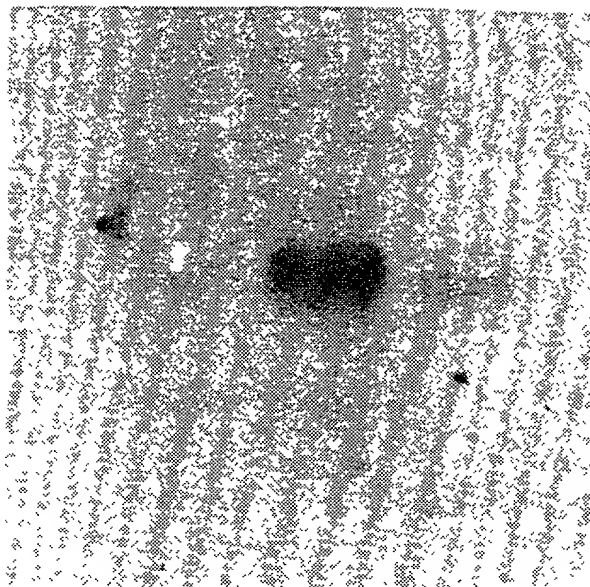


FIG 3F



002190-58925560

4.4 kb—



DAY

5

14

28

FIG. 4

002750-5892550

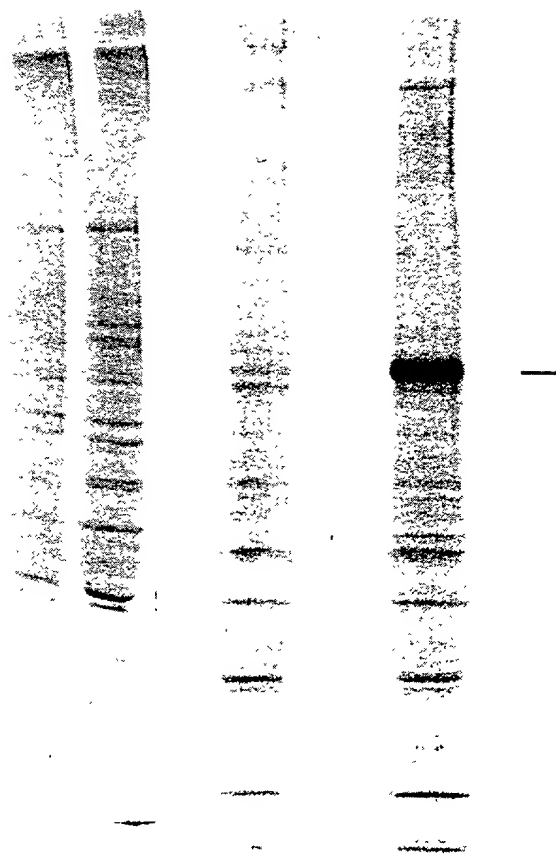


FIG. 5

2026

FIG. 6

ATGAGAGCA CCTCCCCGCG AGGTCTCCGG TGCCACACAGC TCTGCAGCCA CTCTGGCGCC ATGAGAGCGC CGACCACCGC 80
 TCGCTGCTCC GGATGCATCC AACGGGTGCG TTGGAGGGGC TTCTTGCCAC TTGTCTTGGC TGTCTTGATG GGGACAAGTC 160
 ATGCCCAACG GGATTCCATA GGGAGATACG AACCAGTAG CAGGGATGCG AATCGGTTGT GGCACCCCGT GGCAGGCCAC 240
 CCCGCAGCGG CTGCAGCCAA GGTGTACAGT CTGTTCCGAG AGCCTGACGC GCCGGTCCCC GCGTGTGTCG CCTCTGAGTG 320
 GAACCAAGCG GCCCAGGGGA ACCCGGGATG GCTGCGAGAG GCCGAGGCCA GGAGGCCACC TCGAACCCAG CAGCTGCGTC 400
 GAGTCCAGCC ACCTGTCCAG ACTCGGAGAA GCCATCCCCG GGGCCAGCAG CAGATAGCAG CCCGGGCTGC ACCTTCTGTC 480
 GCGCGCCTGG AAACCCCTCA GCGACCCGCG GCTGCACGGC GAGGGCGGCT CACTGGGAGA AATGTCTGCG GGGACACAGT 560
 CTGCCCAGGA TGGACAACAT CAAACAGCAC CAACCACTGT ATCAAAACCTG TGTGTACGCC TCCCCTGTGAG AACCGAGGCT 640
 CCTGCAGCAG GCCCCAGGTC TGCATCTGCC GTTCTGGCTT CCGTGGGGCG CGCTGTGAGG AGGTCAATCCC TGAGGAGGAA 720
 TTTGACCCCTC AGAATGCCAG GCCTGTGCCC AGACGCTCAG TGGAGAGAGC ACCCGGTCTT CACAGAAGCA GTGAGGCCAG 800
 AGGAAAGTCTA GTGACCAGAA TACAGCCGCT GGTACCACCA CCATCACCCAC CTCCATCTCG GCGCCTCAGC CAGCCCTGGC 880
 CCCTGCAGCA GCACTCAGGG CCGTCCAGGA CAGTTCGTGCG GTATCCGGCC ACTGGTGCCA ATGGCCAGCT GATGTCCAAC 960
 GCTTTGGCCTT CAGGACTCGA GCTGAGAGAC AGCAGCCAC AGCAGCACA TGTGAACCAT TGTCAACCC CTCTCACCCC CCTGGGGCT 1040
 GAACCTCACC GAGAAATCA AGAAATCAA AGTCGTCTTC ACCCCACCA TCTGCAAGCA GACCTGTGCC CGGGGACGCT 1120
 GTGCCAACAG CTGTGAGAAG GGTGACACCA CCACCTTGTA CAGTCAGGT GGCCATGGGC ATGACCCCAA GTCTGGCTTC 1200
 CGTATCTATT TCTGCCAAAT CCCCTGCCTG AATGGTGGCC GCTGCATCGG CCGGGACGAG TGCTGGTGTG CAGCCAACTC 1280
 CACAGGAAAG TTCTGCCATC TGCCGTGTCCT GCAGCCAGAC AGGAAACCTG CAGGGCGAGG TTCCCGGCAC AGAACCCCTGC 1360
 TGGAAAGGTC CCTGAAGCAA TCCACCTTCA CGCTGCCCTCT GTGCAGATTG ACCAGTGGC CCGGTCCGG GGTGAGCTGG ACCCCGTGCT 1440
 GTGCAAAATC ATCACCCGCG TGAGGCCTCT CCAGAGCCTC TCATCGCCCC CACGGCAACC TAGGCCACAG CCCCTGGGC AGCAACAGCA 1600
 GGAGGACAAC AGTGTGGAGA CCAGAGCCTC CACCAACAGT GCTGTCTAGG CATTATGGAC TTCTGGGCA GTGTACCTG 1680
 TACCCGCTCG GGCCGGAGAG GCCCTCGGC CAGCAACCTC CTAGGTAGTC TGAATCTCA GGAGGACTGC TGTGGCAGTG TGGGACCTT 1760
 AGCACGGTGA ATGACAGTG TGCTAACCC CTAGGTAGTC TGAATCTCA GGAGGACTGC TGTGGCAGTG TGGGACCTT 1840
 CTGGGGGTG ACCTCCTGTG CTCCCTGCCC ACCAGACAA GAGGGTCCAG CCTTCCAGT GATTGAAAAT GGCCAGCTGG 1840
 AGTGTCCCCA AGGATACAAG AGACTGAAC TCAGCCACTG CCAAGATATC AATGAGTGCC TGACCCCTGGG CCTCTGCAAG 1920

FIG. 7

GACTCGGAGT	GGTGAAACAC	CAGGGGCAGC	TACCTGTGCA	CCTGCAGGCC	TGGCCTCATG	CTGGATCCGT	CAAGGAGCCG	2000
CTGCGTATCG	GACAAAGGCTG	TCTCCATGCA	GCAGGGACTA	TGCTACCGGT	CACTGGGGTC	TGGTACCTGC	ACCCTGCCCTT	2080
TGGTTCATCG	GATCACCAAG	CAGATATGCT	GCTGCAGCCG	TGTGGGCAAA	GCCTGGGGTA	GCACATGTGA	ACAGTGTCCC	2160
CTGCCCTGGCA	CAGAAAGCCTT	CAGGGAGATC	TGCCCTGCTG	GCCATGGCTA	CACCTACTCG	AGCTCAGACA	TCCGCCCTGTC	2240
TATGAGGAAA	GCCGAAGAAG	AGGAACCTGG	TAGCCCCCTTA	AGGAGCAGA	CAGAGCAGAG	CACCTGCACCC	CCACCTGGGC	2320
AAGCAGAGAG	GCAACCACTC	CGGGCAGCCA	CCGCCACCTG	GATTGAGGCT	GAGACCCCTCC	CTGACAAAAGG	TACTCTCGG	2400
GCTGTTTCAGA	TCACAAACCAG	TGCTCCCCAC	CTACCTGCCC	GGGTACCAGG	GGATGCCACT	GGAAGACCAG	CACCATCCTT	2480
GCCTGGACAG	GGCAATCCAG	AGAGTCCAGC	AGAAGAGCAA	GTGATTCCCT	CCAGTGATGT	CTTGGTGACA	CACAGCCCCC	2560
CAGACTTTGA	TCCATGTTTT	GCTGGAGCCT	CCAACATCTG	TGGCCCTGGG	ACCTGTGTGA	GCCTCCCCAAA	TGGATACAGA	2640
TGTGTCCTGCA	GCCCTGGCTA	CCAGCTACAC	CCCAGCCCAAG	ACTACTGTAC	TGATGACAAAC	GAGTGTATGA	GGAACCCCTG	2720
TGAAGGAAGA	GGCGCTGTG	TCAACAGTGT	GGGCTCCTAC	TCTTGCCCTCT	GCTATCCTGG	CTACACACTA	GTCACCCCTCG	2800
GAGACACACA	GGAGTGCCAA	GATATCGATG	AGTGTGAGCA	GCCCCGGGTG	TGCAGTGGTG	GGCGATGCAG	CAACACGGAG	2880
GGCTCGTACC	ACTGCGAGTG	TGATCGGGGC	TACATCATGG	TCAGGAAAGG	ACACTGTCAA	GATATCAACG	AATGCCGTCA	2960
CCCTGGTACC	TGCCCTGATG	GGAGATGCGT	CAACTCCCCCT	GGCTCCTACA	CTTGTCTGGC	CTGTGAGGAG	GGCTATGTAG	3040
GCCAGAGTGG	GAGCTGTGTA	GATGTCAATG	AGTGTCTGAC	CCCTGGGATA	TGTACCCCATG	GAAGGTGCAT	CAACATGGAA	3120
GGCTCCTTTA	GATGCTCCTG	TGAGCCGGGC	TATGAGGTCA	CCCCAGACAA	GAAGGGCTGC	CGAGATGTGG	ACGAGTGTGC	3200
CAGCCGAGCC	TCGTGCCCCA	CGGGCCTCTG	CCTCAACACG	GAGGGCTCCT	TCACCTGCTC	AGCCTGTGAG	AGCGGGTACT	3280
GGGTGAACGA	AGATGGCACT	GCCTGTGAAG	ACTTGGATGA	ATGTGCCCTC	CCTGGAGTCT	GCCCCACAGG	CGTCTGCACC	3360
AATACTGTAG	GCTCCTTCTC	CTGCAAGGAC	TGTGACCAGG	GCTACCGGCC	CAACCCCTTG	GGCAACAGAT	GCGAAGATGT	3440
GGATGAGTGT	GAAGGTCCCC	AAAGCAGCTG	CCGGGGAGGC	GAATGCAAGA	ACACAGAAGG	TTCCCTACCAA	TGCCTCTGTC	3520
ACCAGGGCTT	CCAGCTGGTC	AATGGCACCA	TGTGTGAGGA	CGTGAATGAG	TGTGTTGGGG	AAGAGCATTTG	TGCTCCCTCAC	3600
GGCGAGTGCC	TCAACAGCCT	GGGCTCCTTC	TTCTGCTCTC	GTGCACCCGG	CTTTGCTAGT	GCTGAGGGGG	GCACCAGATG	3680
CCAGGATGTT	GATGAATGTG	CAGCCACAGA	CCCCTGTCCG	GGAGGACACT	GTGTCAACAC	AGAGGGCTCC	TTCAGCTGTC	3760
TGTGTGAGAC	TGCTTTCCTTC	CAGCCCTCCC	CAGACAGCCG	AGAATGTTTG	GATATTGATG	AGTGTGAGGA	CCGTGAAGAC	3840

FIG. 7.1

CCGGTGTGCG GAGCCTGGAG GTGTGAGAAC AGTCCTGGTT CCTACCGCTG CATCCTGGAC TGCCAGCCTG GATTCATGT 3920
 GCGCCAAAT GGAGACTGCA TTGACATAGA TGAATGTGCC AATGACACTG TGTGTGGGAA CCATGGCTTC TGTGACAACA 4000
 CGGACGGCTC CTTCCGCTGC CTGTGTGACC AGGGCTTCGA GACCTCACCA TCAGGCTGGG AGTGTGTTGA TGTGAACGAG 4080
 TGTGAGCTCA TGATGGCAGT GTGTGGGGAT GCGCTCTGTG AGAACGTGGA AGGCTCCTTC CTGTGCCCTTT GCGCCAGTGA 4160
 CCTTGAGGAG TACGACGCAG AAGAAGGACA CTGCCGTCCT CGGGTGGCTG GAGCTCAGAG AATCCCAGAG GTCCGGACAG 4240
 AGGACCAGGC TCCAAGCCTT ATCCGCATGG AATGCTACTC TGAACACAAAT GGTGGTCCCTC CCTGCTCTCA AATCCTGGGC 4320
 CAGAACTCCA CACAGGCCGA GTGCTGCTGC ACTCAGGGTG CCAGATGGGG AAAGGCCTGT GCGCCCTGCC CATCTGAGGA 4400
 CTCAGTTGAA TTCAGTCAGC TCTGCCCCAG TGGTCAAGGT TACATCCCAG TGGAAAGGAGC CTGGACATTT GGACAAACCA 4480
 TGTATACAGA TGCCGATGAA TGTGTACTGT TTGGGCCCTGC TCTCTGCCAG AATGGCCGAT GCTCAAAACAT AGTGCCCTGGC 4560
 TACATTTGCC TGTGCAACCC TGGCTACCAC TATGATGCCT CCAGCAGGAA GTGCCCAGGAT CACAACGAAT GCCAGGACTT 4640
 GGCCTGTGAG AACGGTGAGT GTGTGAACCA AGAAGGCTCC TTCCATTGCC TCTGCAATCC CCCCCTCACC CTAGACCTCA 4720
 GTGGGCAGCG CTGTGTGAAC ACGACCAGCA GCACGGAGGA CTTCCTTGAC CATGACATCC ACATGGACAT CTGCTGGAAA 4800
 AAAGTCACCA ATGATGTGTG CAGCCAGCCC TTGCGTGGGC ACCATACCAC CTATACAGAA TGCTGCTGCC AAGATGGGA 4880
 GGCCTGGAGC CAGCAATGCG CTCTGTGCCC GCCCAGGAGC TCTGAGTCT ACGCTCAGCT GTGCAACGTG GCTCGGATTG 4960
 AGGCAGAGCG CGGAGCAGGG ATCCACTTCC GGCCAGGCTA TGAGTATGGC CCTGGCCTGG ACGATCTGCC TGAAAACCTC 5040
 TACGGCCCG ATGGGGCTCC CTTCTATAAC TACCTAGGCC CCGAGGACAC TGCCCCCTGAG CCTCCCTTCT CCAACCCAGC 5120
 CAGCCAGCCG GGAGACAACA CACCTGTCCT TGAGCCTCCT CTGCAGCCCCT CTGAACCTCA GCCTCACTAT CTAGCCAGCC 5200
 ACTCAGAACC CCTTGCCCTCC TTCGAAGGCC TTCAGGCTGA GGAATGTGGC ATCCTGAATG GCTGTGAGAA TGGCCGCTGC 5280
 GTGCGTGTGC GGGAGGGCTA CACTTGCGAC TGCTTTGAGG GCTTCCAGCT GGATGCGCCC ACATTTGGCT GTGTGGATGT 5360
 GAACGAGTGT GAAGACTTGA ACGGGCCTGC ACGACTCTGT GCACACGGTC ACTGTGAGAA CACAGAGGGT TCCTATCGCT 5440
 GCCACTGTTT GCCAGGTTAC GTGGCAGAGC CAGGCCCCCC ACACTGTGCG GCCAAGGAGT AG 5502

FIG. 7.2

90 MESTSPRLRCPQLCSHSGAMRAPTTARCSGCIQVRWRGFLPLVLAVLMGTSHAQORDSIGRYEPASRDANRLWHPVGSHPAAAAKVYS
 180 LFREPDAPVPLSPSEWNQPAQGNPGWLAAEAERPPRTQQLLRRVQPPVQTRRSHPRGQQQIAARAAAPSVARLETTPQRPAAARRGRLTGR
 270 NVCGGCCPGWTTNSTNHCIKPVCQPPCQNRGSCSRPQVCICRSGFRGARCEEVIPLEEFDPQONARVPPRRSVERAPGPHRSSEARGSL
 360 VTRIQLPLVPPPPPPRRRLSQPWPQLQHSGPSRTVRRYPATGANGQLMSNALPSGLELRDSSPQAAHVNLHSPWGLNLTEKIKIKVVF
 450 TPTICKQTCARGRCANSCCKGDTTTLYSQGGHGDHPKSGFRIYFCQIPCLNGGRCIGRDECWCPCANSTGKFKCHLPVPQPDREPAGRSRH
 540 RTLLEGPLKQSTFTLPLSNQLASVNPISLVKVQIHQPVARVRGELDPVLEDNSVETRASHRPHGNLGHSPWASNSIPARAGE
 630 APRPPVLSRHYGLLGQCYLSTVNGQCANPLGSLTSQEDCCGSVGTFWGVTSCAPCPRQEGPAFPVIENGQLECPQGYKRLNLSHCQDI
 720 NECLTLGLCKDSECVNTRGSYLCTCRPGLMLDPSRRCVSDKAVSMQGLCYRSLGSGTCTLPLVHRITKQICCCSRVGKAWGSTCEQCP
 810 LPGTEAFREI CPAGHGYTYSSDIRLSMRKAEELASPLREQTEQSTAPPQGAERQPLRAATATWIEAETLPDKGDSRAVQITTSAPH
 900 LPARVPGDATGRPAPSLPGQGIPESPAEEQVIPSSDVLVTHSPPDFPCFAGASNICGPGTVCVSLPNGYRCVCSPGYQLHPSQDYCTDDN
 990 ECMRNPCEGRGRCVNSVGSYCLCYPGYTLVTLGDTQECQDIDECQPGVCSGGRCNSTEGSYHCECDRGYIMVRKGHCQDINECRHPGT
 1080 CPDGRCVNSPGSYTCLACEEGYVQSGSCVDVNECLTPGICTHGRGINMEGSFRCSCEPGYEVTPDKKGRDVEDCASRASCTGLCLNT
 1170 EGSFTCSACQSGYWVNEDGTACEDLDECAFPVCPTGVTNTVGSFCKDCDQGYRPNPLGNRCEDVDECEGPQSSCRGGECKNTEGSYQ
 1260 CLCHQGFQLVNGTMCEDVNECVGEEHCAPHGECNLSLGSFFCLCAPGFASAEGGTRCQDVDECAATDPCPGGHCVNTEGSFSLCETASF
 1350 QPSPDSGECLDIDECEDREDPVCGAWRCENSPGSYRCILDCQPGFYVAPNGDCIDIDECANDTVCGNHGFCNDTDGSRCLCDQGFETSP
 1440 SGWECVDVNECELMMAVCGDALCENVEGSFLCLCASDLEEYDAEEGHCPRVAGAQRIPVETEDQAPSLIRMECYSEHNGGPPCSQILG
 1530 QNSTQAECCCTQGARWGKACAPCPSEDSVEFSQLCPSGQGYIPVEGAWTFGQTM'TDADECVLFGPALCQNGRCSNIVPGYICLCNPGYH
 1620 YDASSRKQDHNQCQDLACENGECVNQEGSFHCLCNPPPLTLDLSGQRCVNTTSSSTEDFPDHDIHMDICWKKVTNDVCSQPLRGHHTTYTE
 1710 CCCQDGEAWSQQCALCPPRSSEVYAQLCNVARIEAERGAGIHFRPGYEGPGLDDLPENLYGPDGAPFYNYLGPEDTAPEPPFSNPASQP
 1800 GDNTPVLEPPLQPSLQPHYLAHSEPPASFEGLOAECCGILNGCENGRCVRREGYTCDCFEQFQLDAPTILACVDVNECEDLNGPARLC
 1833 AHGHCENTEGSYRCHCSPGYVAEPGPPHCAAKE

FIG. 8

ATG	CGC	CAG	GCC	GCA	TTG	GGG	CTG	CTG	GCA	CTA	CTC	CTG	CTG	GCG	CTG	CTG	GGC	54
M	R	Q	A	A	L	G	L	L	A	L	L	L	L	A	L	L	G	18
CCC	GGC	GGC	CGA	GGG	GTG	GGC	CGG	CCG	GGC	AGC	GGG	GCA	CAG	GCG	GGG	GCG	GGG	108
P	G	G	R	G	V	G	R	P	G	S	G	A	Q	A	G	A	G	36
CGC	TGG	GCC	CAA	CGC	TTC	AAG	GTG	GTC	TTT	GCG	CCT	GTG	ATC	TGC	AAG	CGG	ACC	162
R	W	A	Q	R	F	K	V	V	F	A	P	V	I	C	K	R	T	54
TGT	CTG	AAG	GGC	CAG	TGT	CGG	GAC	AGC	TGT	CAG	CAG	GGC	TCC	AAC	ATG	ACG	CTC	216
C	L	K	G	Q	C	R	D	S	C	Q	Q	G	S	<u>N</u>	<u>M</u>	<u>T</u>	L	72
ATC	GGA	GAG	AAC	GGC	CAC	AGC	ACC	GAC	ACG	CTC	ACC	GGT	TCT	GCC	TTC	CGC	GTG	270
I	G	E	N	G	H	S	T	D	T	L	T	G	S	A	F	R	V	90
GTG	GTG	TGC	CCT	CTA	CCC	TGC	ATG	AAC	GGT	GGC	CAG	TGC	TCT	TCC	CGA	AAC	CAG	324
V	V	C	P	L	P	C	M	N	G	G	Q	C	S	S	R	N	Q	108
TGC	CTG	TGT	CCC	CCG	GAT	TTC	ACG	GGG	CGC	TTC	TGC	CAG	GTG	CCT	GCT	GCA	GGA	378
C	L	C	P	P	D	F	T	G	R	F	C	Q	V	P	A	A	G	126
ACC	GGA	GCT	GGC	ACC	GGG	AGT	TCA	GGC	CCC	GGC	TGG	CCC	GAC	CGG	GCC	ATG	TCC	432
T	G	A	G	T	G	S	S	G	P	G	W	P	D	R	A	M	S	144
ACA	GGC	CCG	CTG	CCG	CCC	CTT	GCC	CCA	GAA	GGA	GAG	TCT	GTG	GCT	AGC	AAA	CAC	486
T	G	P	L	P	P	L	A	P	E	G	E	S	V	A	S	K	H	162
GCC	ATT	TAC	GCG	GTG	CAG	GTG	ATC	GCA	GAT	CCT	CCC	GGG	CCG	GGG	GAG	GGT	CCT	540
A	I	Y	A	V	Q	V	I	A	D	P	P	G	P	G	E	G	P	180
CCT	GCA	CAA	CAT	GCA	GCC	TTC	TTG	GTG	CCC	CTG	GGG	CCA	GGA	CAA	ATC	TCG	GCA	594
P	A	Q	H	A	A	F	L	V	P	L	G	P	G	Q	I	S	A	198
GAA	GTG	CAG	GCT	CCG	CCC	CCC	GTG	GTG	AAC	GTG	CGT	GTC	CAT	CAC	CCT	CCT	GAA	648
E	V	Q	A	P	P	P	V	V	N	V	R	V	H	H	P	P	E	216
GCT	TCC	GTT	CAG	GTG	CAC	CGC	ATC	GAG	GGG	CCG	AAC	GCT	GAA	GGC	CCA	GCC	TCT	702
A	S	V	Q	V	H	R	I	E	G	P	N	A	E	G	P	A	S	234
TCC	CAG	CAC	TTG	CTG	CCG	CAT	CCC	AAG	CCC	CAG	CAC	CCG	AGG	CCA	CCC	ACT	CAA	756
S	Q	H	L	L	P	H	P	K	P	Q	H	P	R	P	P	T	Q	252
AAG	CCA	CTG	GGC	CGC	TGC	TTC	CAG	GAC	ACA	TTG	CCC	AAG	CAG	CCT	TGT	GGC	AGC	810
K	P	L	G	R	C	F	Q	D	T	L	P	K	Q	P	C	G	S	270
AAC	CCT	TTG	CCT	GGC	CTT	ACC	AAG	CAG	GAA	GAT	TGC	TGC	GGT	AGC	ATC	GGT	ACT	864
N	P	L	P	G	L	T	K	Q	E	D	C	C	G	S	I	G	T	288
GCC	TGG	GGA	CAA	AGC	AAG	TGT	CAC	AAG	TGC	CCA	CAG	CTT	CAG	TAT	ACA	GGG	GTG	918
A	W	G	Q	S	K	C	H	K	C	P	Q	L	Q	Y	T	G	V	306
CAG	AAG	CCT	GTA	CCT	GTA	CGT	GGG	GAG	GTG	GGT	GCT	GAC	TGC	CCC	CAG	GGC	TAC	972
Q	K	P	V	P	V	R	G	E	V	G	A	D	C	P	Q	G	Y	324
AAG	AGG	CTC	AAC	AGC	ACC	CAC	TGC	CAG	GAT	ATC	AAC	GAA	TGT	GCG	ATG	CCC	GGG	1026
K	R	L	<u>N</u>	<u>S</u>	<u>T</u>	H	C	Q	D	I	N	E	C	A	M	P	G	342

FIG. 9

AAT	GTG	TGC	CAT	GGT	GAC	TGC	CTC	AAC	AAC	CCT	GGC	TCT	TAT	CGC	TGT	GTC	TGC	1080
N	V	C	H	G	D	C	L	N	N	P	G	S	Y	R	C	V	C	360
CCG	CCC	GGT	CAT	AGC	TTG	GGT	CCC	CTC	GCA	GCA	CAG	TGC	ATT	GCC	GAC	AAA	CCA	1134
P	P	G	H	S	L	G	P	L	A	A	Q	C	I	A	D	K	P	378
GAG	GAG	AAG	AGC	CTG	TGT	TTC	CGC	CTT	GTG	AGC	ACC	GAA	CAC	CAG	TGC	CAG	CAC	1188
E	E	K	S	L	C	F	R	L	V	S	T	E	H	Q	C	Q	H	396
CCT	CTG	ACC	ACA	CGC	CTA	ACC	CGC	CAG	CTC	TGC	TGC	TGT	AGT	GTG	GGT	AAA	GCC	1242
P	L	T	T	R	L	T	R	Q	L	C	C	C	S	V	G	K	A	414
TGG	GGT	GCC	CGG	TGC	CAG	CGC	TGC	CCG	GCA	GAT	GGT	ACA	GCA	GCC	TTC	AAG	GAG	1296
W	G	A	R	C	Q	R	C	P	A	D	G	T	A	A	F	K	E	432
ATC	TGC	CCC	GGC	TGG	GAA	AGG	GTA	CCA	TAT	CCT	CAC	CTC	CCA	CCA	GAC	GCT	CAC	1350
I	C	P	G	W	E	R	V	P	Y	P	H	L	P	P	D	A	H	450
CAT	CCA	GGG	GGA	AAG	CGA	CTT	CTC	CCT	CTT	CCT	GCA	CCC	GAC	GGG	CCA	CCC	AAA	1404
H	P	G	G	K	R	L	L	P	L	P	A	P	D	G	P	P	K	468
CCC	CAG	CAG	CTT	CCT	GAA	AGC	CCC	AGC	CGA	GCA	CCA	CCC	CTC	GAG	GAC	ACA	GAG	1458
P	Q	Q	L	P	E	S	P	S	R	A	P	P	L	E	D	T	E	486
GAA	GAG	AGA	GGA	GTG	ACC	ATG	GAT	CCA	CCA	GTG	AGT	GAG	GAG	CGA	TCG	GTG	CAG	1512
E	E	R	G	V	T	M	D	P	P	V	S	E	E	R	S	V	Q	504
CAG	AGC	CAC	CCC	ACT	ACC	ACC	ACC	TCA	CCC	CCC	CGG	CCT	TAC	CCA	GAG	CTC	ATC	1566
Q	S	H	P	T	T	T	T	S	P	P	R	P	Y	P	E	L	I	522
TCT	CGC	CCC	TCC	CCA	CCT	ACC	TTC	CAC	CGG	TTC	CTG	CCA	GAC	TTG	CCC	CCA	TCC	1620
S	R	P	S	P	P	T	F	H	R	F	L	P	D	L	P	P	S	540
CGA	AGT	GCA	GTG	GAG	ATC	GCC	CCC	ACT	CAG	GTC	ACA	GAG	ACC	GAT	GAG	TGC	CGA	1674
R	S	A	V	E	I	A	P	T	Q	V	T	E	T	D	E	C	R	558
TTG	AAC	CAG	AAT	ATC	TGT	GGC	CAT	GGA	CAG	TGT	GTG	CCT	GGC	CCC	TCG	GAT	TAC	1728
L	N	Q	N	I	C	G	H	G	Q	C	V	P	G	P	S	D	Y	576
TCC	TGC	CAC	TGC	AAC	GCT	GGC	TAC	CGG	TCA	CAC	CCG	CAG	CAC	CGC	TAC	TGT	GTT	1782
S	C	H	C	N	A	G	Y	R	S	H	P	Q	H	R	Y	C	V	594
GAT	GTG	AAC	GAG	TGC	GAG	GCA	GAG	CCC	TGC	GGC	CCC	GGG	AAA	GGC	ATC	TGT	ATG	1836
D	V	N	E	C	E	A	E	P	C	G	P	G	K	G	I	C	M	612
AAC	ACT	GGT	GGC	TCC	TAC	AAT	TGT	CAC	TGC	AAC	CGA	GGC	TAC	CGC	CTC	CAC	GTG	1890
N	T	G	G	S	Y	N	C	H	C	N	R	G	Y	R	L	H	V	630
GGT	GCA	GGG	GGC	CGC	TCG	TGC	GTG	GAC	CTG	AAC	GAG	TGC	GCC	AAG	CCT	CAC	CTG	1944
G	A	G	G	R	S	C	V	D	L	N	E	C	A	K	P	H	L	648
TGT	GGG	GAC	GGT	GGC	TTC	TGC	ATC	AAC	TTC	CCT	GGT	CAC	TAC	AAA	TGC	AAC	TGC	1998
C	G	D	G	G	F	C	I	N	F	P	G	H	Y	K	C	N	C	666
TAT	CCT	GGC	TAC	CGG	CTC	AAG	GCC	TCC	CGA	CCG	CCC	ATT	TGC	GAA	GAC	ATC	GAC	2052
Y	P	G	Y	R	L	K	A	S	R	P	P	I	C	E	D	I	D	684
GAG	TGT	CGC	GAC	CCT	AGC	ACC	TGC	CCT	GAT	GGC	AAA	TGT	GAA	AAC	AAA	CCT	GGC	2106
E	C	R	D	P	S	T	C	P	D	G	K	C	E	N	K	P	G	702

FIG. 9.1

AGC	TTC	AAG	TGC	ATC	GCC	TGC	CAG	CCT	GGC	TAC	CGT	AGC	CAG	GGG	GGC	GGG	GCC	2160
S	F	K	C	I	A	C	Q	P	G	Y	R	S	Q	G	G	G	A	720
TGT	CGT	GAT	GTC	AAC	GAA	TGC	TCC	GAA	GGT	ACC	CCC	TGC	TCT	CCT	GGA	TGG	TGT	2214
C	R	D	V	N	E	C	S	E	G	T	P	C	S	P	G	W	C	738
GAG	AAA	CTT	CCG	GGT	TCT	TAC	CGT	TGC	ACG	TGT	GCC	CAG	GGG	ATA	CGA	ACC	CGC	2268
E	K	L	P	G	S	Y	R	C	T	C	A	Q	G	I	R	T	R	756
ACA	GGA	CGC	CTC	AGT	TGC	ATA	GAC	GTG	GAT	GAC	TGT	GAG	GCT	GGG	AAA	GTG	TGC	2322
T	G	R	L	S	C	I	D	V	D	D	C	E	A	G	K	V	C	774
CAA	GAT	GGC	ATC	TGC	ACG	AAC	ACA	CCA	GGC	TCT	TTC	CAG	TGT	CAG	TGC	CTC	TCC	2376
Q	D	G	I	C	T	N	T	P	G	S	F	Q	C	Q	C	L	S	792
GGC	TAT	CAT	CTG	TCA	AGG	GAT	CGG	AGC	CGC	TGT	GAG	GAC	ATT	GAT	GAA	TGT	GAC	2430
G	Y	H	L	S	R	D	R	S	R	C	E	D	I	D	E	C	D	810
TTC	CCT	GCG	GCC	TGC	ATC	GGG	GGT	GAC	TGC	ATC	AAT	ACC	AAT	GGT	TCC	TAC	AGA	2484
F	P	A	A	C	I	G	G	D	C	I	N	T	<u>N</u>	<u>G</u>	<u>S</u>	Y	R	828
TGT	CTC	TGT	CCC	CTG	GGT	CAT	CGG	TTG	GTG	GGC	GGC	AGG	AAG	TGC	AAG	AAA	GAT	2538
C	L	C	P	L	G	H	R	L	V	G	G	R	K	C	K	K	D	846
ATA	GAT	GAG	TGC	AGC	CAG	GAC	CCA	GGC	CTG	TGC	CTG	CCC	CAT	GCC	TGC	GAG	AAC	2592
I	D	E	C	S	Q	D	P	G	L	C	L	P	H	A	C	E	N	864
CTC	CAG	GGC	TCC	TAT	GTC	TGT	GTC	TGT	GAT	GAG	GGT	TTC	ACA	CTC	ACC	CAG	GAC	2646
L	Q	G	S	Y	V	C	V	C	D	E	G	F	T	L	T	Q	D	882
CAG	CAT	GGG	TGT	GAG	GAG	GTG	GAG	CAG	CCC	CAC	CAC	AAG	AAG	GAG	TGC	TAC	CTT	2700
Q	H	G	C	E	E	V	E	Q	P	H	H	K	K	E	C	Y	L	900
AAC	TTC	GAT	GAC	ACA	GTG	TTC	TGT	GAC	AGC	GTA	TTG	GCT	ACC	AAT	GTC	ACT	CAG	2754
N	F	D	D	T	V	F	C	D	S	V	L	A	T	<u>N</u>	<u>V</u>	<u>T</u>	Q	918
CAG	GAA	TGC	TGT	TGC	TCT	CTG	GGA	GCT	GGC	TGG	GGA	GAC	CAC	TGC	GAA	ATC	TAT	2808
Q	E	C	C	C	S	L	G	A	G	W	G	D	H	C	E	I	Y	936
CCC	TGT	CCA	GTC	TAC	AGC	TCA	GCC	GAA	TTT	CAC	AGC	CTG	GTG	CCT	GAT	GGG	AAA	2862
P	C	P	V	Y	S	S	A	E	F	H	S	L	V	P	D	G	K	954
AGG	CTA	CAC	TCA	GGA	CAA	CAA	CAT	TGT	GAA	CTA	TGC	ATT	CCT	GCC	CAC	CGT	GAC	2916
R	L	H	S	G	Q	Q	H	C	E	L	C	I	P	A	H	R	D	972
ATC	GAC	GAA	TGC	ATA	TTG	TTT	GGG	GCA	GAG	ATC	TGC	AAG	GAG	GGC	AAG	TGT	GTG	2970
I	D	E	C	I	L	F	G	A	E	I	C	K	E	G	K	C	V	990
AAC	TCG	CAG	CCC	GGC	TAC	GAG	TGC	TAC	TGC	AAG	CAG	GGC	TTC	TAC	TAC	GAT	GGC	3024
N	S	Q	P	G	Y	E	C	Y	C	K	Q	G	F	Y	Y	D	G	1008
AAC	CTG	CTG	GAG	TGC	GTG	GAC	GTG	GAC	GAG	TGC	TTG	GAT	GAG	TCT	AAC	TGC	AGG	3078
N	L	L	E	C	V	D	V	D	E	C	L	D	E	S	N	C	R	1026
AAC	GGA	GTG	TGT	GAG	AAC	ACG	TGG	CGG	CTA	CCG	TGT	GCC	TGC	ACT	CCG	CCG	GCA	3132
N	G	V	C	E	N	T	W	R	L	P	C	A	C	T	P	P	A	1044
GAG	TAC	AGT	CCC	GCA	CAG	GCC	CAG	TGT	CTG	AGC	CCG	GAG	GAG	ATG	GAG	CAC	GCC	3186
E	Y	S	P	A	Q	A	Q	C	L	S	P	E	E	M	E	H	A	1062

CCA GAG AGA CGT GAA GTG TGC TGG GGC CAG CGA GGA GAG GAC GGC ATG TGT ATG 3240
 P E R R E V C W G Q R G E D G M C M 1080
 GGG CCC CTG GCG GGA CCT GCC CTC ACT TTT GAT GAC TGC TGC TGC CGC CAG CCG 3294
 G P L A G P A L T F D D C C C R Q P 1098
 CGG CTG GGG TAC CAG TGC AGA CCG TGC CCG CCA CGT GGC ACC GGG TGC CAG TGC 3348
 R L G Y Q C R P C P P R G T G S Q C 1116
 CCG ACT TCA CAG AGT GAG AGC AAT TCT TTC TGG GAC ACA AGC CCC CTG CTA CTG 3402
 P T S Q S E S N S F W D T S P L L L 1134
 GGG AAG TCT CCG CGA GAC GAA GAC AGC TCA GAG GAG GAT TCA GAT GAG TGC CGT 3456
 G K S P R D E D S S E E D S D E C R 1152
 TGT GTG AGC GGA CCG TGT GTG CCA CGG CCA GGC GGG GCG GTA TGC GAG TGT CCT 3510
 C V S G P C V P R P G G A V C E C P 1170
 GGA GGC TTT CAG CTG GAC GCC TCC CGT GCC CGC TGC GTG GAC ATT GAT GAG TGC 3564
 G G F Q L D A S R A R C V D I D E C 1188
 CGA GAA CTG AAC CAG CGG GGA CTG CTG TGT AAG AGC GAG CGG TGC GTG AAC ACC 3618
 R E L N Q R G L L C K S E R C V N T 1206
 AGT GGA TCC TTC CGC TGT GTC TGC AAA GCT GGC TTC ACG CGC AGC CGC CCT CAC 3672
S G S F R C V C K A G F T R S R P H 1224
 GGG CCT GCG TGC CTC AGC GCC GCC GCT GAT GAT GCA GCC ATA GCC CAC ACC TCA 3726
 G P A C L S A A A D D A A I A H T S 1242
 GTG ATC GAT CAT CGA GGG TAT TTT CAC TGA
 V I D H R G Y F H *

FIG. 9.3

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Met Arg Gln Ala Ala Leu Gly Leu Gly Leu Ala Leu Leu Leu Ala Leu Leu Gly Leu Gly Pro Gly Gly Arg	22
Gly Val Gly Arg Pro Gly Ser Gly Ala Gln Ala Gly Ala Gly Arg Trp Ala Gln Arg Phe Lys Val	44
Val Phe Ala Pro Val Ile Cys Lys Arg Thr Cys Leu Lys Ser Thr Asp Thr Cys Arg Asp Ser Cys Gln Gln	66
Gly Ser Asn Met Thr Leu Ile Gly Glu Asn Gly His Ser Thr Asp Thr Leu Thr Gly Ser Ala Phe	88
Arg Val Val Val Cys Pro Leu Pro Cys Met Asn Gly Gly Gln Cys Ser Ser Arg Asn Gln Cys Leu	110
Cys Pro Pro Asp Phe Thr Gly Arg Phe Cys Gln Val Pro Ala Ala Gly Thr Gly Ala Gly Thr Gly	132
Ser Ser Gly Pro Gly Trp Pro Asp Arg Ala Met Ser Thr Gly Pro Gly Pro Leu Pro Leu Ala Pro Glu	154
Gly Glu Ser Val Ala Ser Lys His Ala Ile Tyr Ala Val Gln Val Ile Ala Asp Pro Gly Pro	176
Gly Glu Gly Pro Pro Ala Gln His Ala Ala Phe Leu Val Pro Leu Gly Pro Gly Gln Ile Ser Ala	198
Glu Val Gln Ala Pro Pro Val Val Asn Val Arg Val His His Pro Pro Glu Ala Ser Val Gln	220
Val His Arg Ile Glu Gly Pro Asn Ala Glu Gly Pro Ala Ser Ser Gln His Leu Leu Pro His Pro	242
Lys Pro Pro His Pro Arg Pro Thr Gln Lys Pro Leu Gly Arg Cys Phe Gln Asp Thr Leu Pro	264
Lys Gln Pro Cys Gly Ser Asn Pro Leu Pro Gly Leu Thr Lys Gln Glu Asp Cys Cys Gly Ser Ile	286
Gly Thr Ala Trp Gly Gln Ser Lys Cys His Lys Cys Pro Gln Leu Gln Tyr Thr Gly Val Gln Lys	308
Pro Val Pro Val Arg Gly Glu Val Gly Ala Asp Cys Pro Gln Gly Tyr Lys Arg Leu Asn Ser Thr	330
His Cys Gln Asp Ile Asn Glu Cys Ala Met Pro Gly His Ser Leu Gly Pro Leu Ala Ala Gln Cys Ile	352
Pro Gly Ser Tyr Arg Cys Val Cys Pro Gly His Ser Leu Gly Pro Leu Ala Ala Gln Cys Ile	374
Ala Asp Lys Pro Glu Glu Lys Ser Arg Phe Cys Cys Ser Val Gly Lys Ala Trp Gly Ala Arg	396
Pro Leu Thr Thr Arg Leu Thr Arg Gln Leu Cys Phe Arg Leu Val Ser Thr Glu His Gln Cys Gln His	418
Cys Gln Arg Cys Pro Ala Asp Gly Thr Ala Ala Phe Lys Glu Ile Cys Pro Gly Trp Glu Arg Val	440
Pro Tyr Pro His Leu Pro Pro Asp Ala His His Pro Gly Gly Lys Arg Leu Leu Pro Leu Pro Ala	462
Pro Asp Gly Pro Pro Lys Pro Gln Gln Leu Pro Glu Ser Pro Ser Arg Ala Pro Pro Leu Glu Asp	484

FIG. 10

Thr Glu Glu Glu Arg Gly Val Thr Met Asp Pro Pro Val Ser Glu Glu Arg Ser Val Gln Gln Ser 506
 His Pro Thr Thr Thr Ser Ser Pro Pro Arg Pro Tyr Pro Glu Leu Ile Ser Arg Pro Ser Pro Pro 528
 Thr Phe His Arg Phe Leu Pro Asp Leu Pro Pro Ser Arg Ser Ala Val Glu Ile Ala Pro Thr Gln 550
 Val Thr Glu Thr Asp Glu Cys Arg Leu Asn Gln Asn Ile Cys Gly His Gly Gln Cys Val Pro Gly 572
 Pro Ser Asp Tyr Ser Cys His Cys Asn Ala Gly Tyr Arg Ser His Pro Gln His Arg Tyr Cys Val 594
 Asp Val Asn Glu Cys Glu Ala Glu Pro Cys Gly Pro Gly Lys Gly Ile Cys Met Asn Thr Gly Gly 616
 Ser Tyr Asn Cys His Cys Asn Arg Gly Tyr Arg Leu His Val Gly Ala Gly Arg Ser Cys Val 638
 Asp Leu Asn Glu Cys Ala Lys Pro His Leu Cys Gly Asp Gly Gly Phe Cys Ile Asn Phe Pro Gly 660
 His Tyr Lys Cys Asn Cys Tyr Pro Gly Tyr Arg Leu Lys Ala Ser Arg Pro Pro Ile Cys Glu Asp 682
 Ile Asp Glu Cys Arg Asp Pro Ser Thr Cys Pro Asp Gly Lys Cys Glu Asn Lys Pro Gly Ser Phe 704
 Lys Cys Ile Ala Cys Gln Pro Gly Tyr Arg Ser Gln Gly Gly Ala Cys Arg Asp Val Asn Glu 726
 Cys Ser Glu Gly Thr Pro Cys Ser Pro Gly Trp Cys Glu Lys Leu Pro Gly Ser Tyr Arg Cys Thr 748
 Cys Ala Gln Gly Ile Arg Thr Arg Thr Gly Arg Leu Ser Cys Ile Asp Val Asp Cys Glu Ala 770
 Gly Lys Val Cys Gln Asp Gly Ile Cys Thr Asn Thr Pro Gly Ser Phe Gln Cys Gln Cys Leu Ser 792
 Gly Tyr His Leu Ser Arg Asp Arg Ser Arg Cys Glu Asp Ile Asp Glu Cys Asp Phe Pro Ala Ala 814
 Cys Ile Gly Gly Asp Cys Ile Asn Thr Asn Gly Ser Tyr Arg Cys Pro Leu Gly His Arg 836
 Leu Val Gly Gly Arg Lys Cys Lys Lys Asp Ile Asp Glu Cys Ser Gln Asp Pro Gly Leu Cys Leu 858
 Pro His Ala Cys Glu Asn Leu Gln Gly Ser Tyr Val Cys Val Cys Asp Glu Gly Phe Thr Leu Thr 880
 Gln Asp Gln His Gly Cys Glu Glu Val Leu Ala Thr Asn Val Thr Lys Lys Glu Cys Tyr Leu Asn Phe 902
 Asp Asp Thr Val Phe Cys Asp Ser Val Leu Leu Ala Thr Asn Val Thr Gln Gln Glu Cys Cys Ser 924
 Leu Gly Ala Gly Trp Gly Asp His Cys Glu Ile Tyr Pro Cys Pro Val Tyr Ser Ser Ala Glu Phe 946
 His Ser Leu Val Pro Asp Gly Lys Arg Leu His Ser Gly Gln Gln His Cys Glu Leu Cys Ile Pro 968

FIG. 10.1

Ala	His	Arg	Asp	Ile	Asp	Glu	Cys	Ile	Leu	Phe	Gly	Ala	Glu	Ile	Cys	Lys	Glu	Gly	Lys	Cys	Val	990
Asn	Ser	Gln	Pro	Gly	Tyr	Glu	Cys	Tyr	Cys	Lys	Gln	Gly	Phe	Tyr	Tyr	Asp	Gly	Asn	Leu	Leu	Glu	1012
Cys	Val	Asp	Val	Asp	Glu	Cys	Leu	Asp	Glu	Ser	Asn	Cys	Arg	Asn	Gly	Val	Cys	Glu	Asn	Thr	Trp	1034
Arg	Leu	Pro	Cys	Ala	Cys	Thr	Pro	Pro	Ala	Glu	Tyr	Ser	Pro	Ala	Gln	Ala	Gln	Cys	Leu	Ile	Pro	1056
Glu	Arg	Trp	Ser	Thr	Pro	Gln	Arg	Asp	Val	Lys	Cys	Ala	Gly	Ala	Ser	Glu	Glu	Arg	Thr	Ala	Cys	1078
Val	Trp	Gly	Pro	Trp	Ala	Gly	Pro	Ala	Leu	Thr	Phe	Asp	Asp	Cys	Cys	Cys	Arg	Gln	Pro	Arg	Leu	1100
Gly	Thr	Gln	Cys	Arg	Pro	Cys	Pro	Pro	Arg	Gly	Thr	Gly	Ser	Gln	Cys	Pro	Thr	Ser	Gln	Ser	Glu	1122
Ser	Asn	Ser	Phe	Trp	Asp	Thr	Ser	Pro	Leu	Leu	Gly	Gly	Lys	Ser	Pro	Arg	Asp	Glu	Asp	Ser	Ser	1144
Glu	Glu	Asp	Ser	Asp	Glu	Cys	Arg	Cys	Val	Ser	Gly	Pro	Cys	Val	Pro	Arg	Pro	Gly	Gly	Ala	Val	1166
Cys	Glu	Cys	Pro	Gly	Gly	Phe	Gln	Leu	Asp	Ala	Ser	Arg	Ala	Arg	Cys	Val	Asp	Ile	Asp	Glu	Cys	1188
Arg	Glu	Leu	Asn	Gln	Arg	Gly	Leu	Leu	Cys	Lys	Ser	Glu	Arg	Cys	Val	Asn	Thr	Ser	Gly	Ser	Phe	1210
Arg	Cys	Val	Cys	Lys	Ala	Gly	Phe	Thr	Arg	Ser	Arg	Pro	His	Gly	Pro	Ala	Cys	Leu	Ser	Ala	Ala	1232
Ala	Asp	Asp	Ala	Ala	Ile	Ala	His	Thr	Ser	Val	Ile	Asp	His	Arg	Gly	Tyr	Phe	His				1251

—

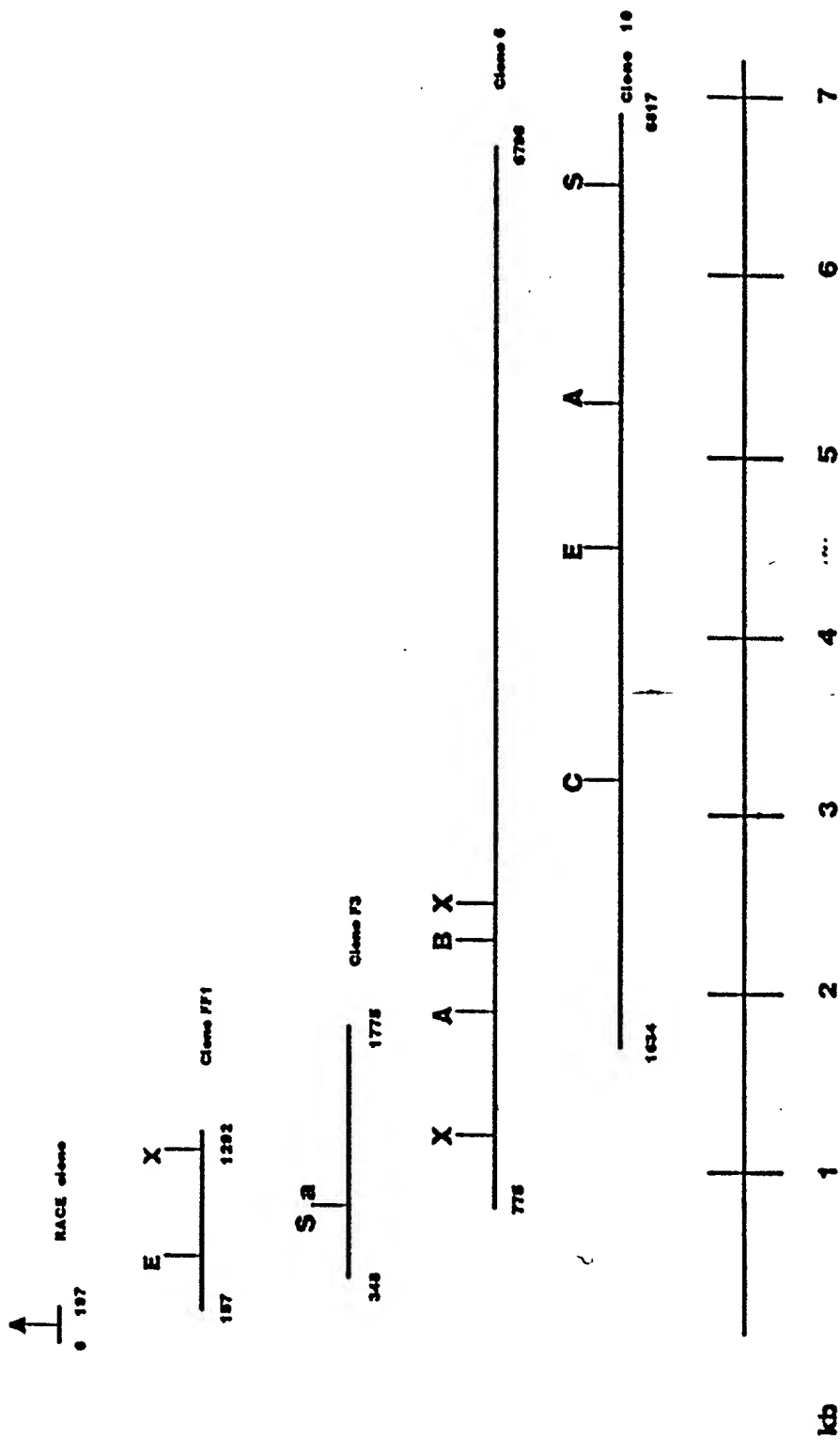


FIG. 11

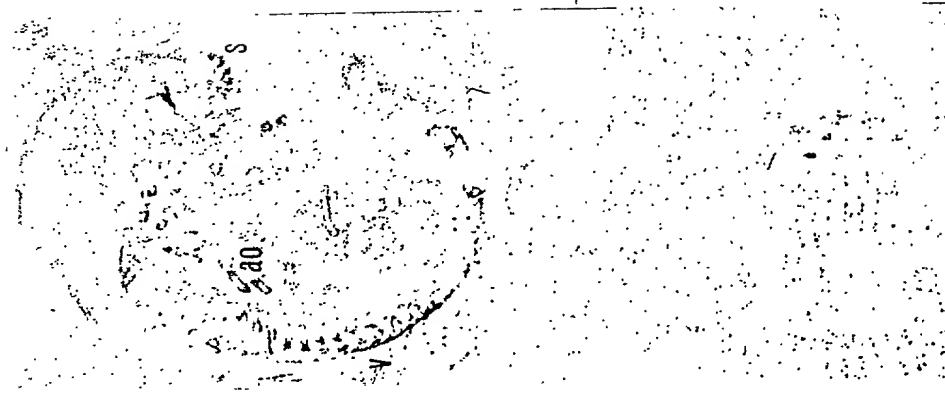


FIG. 12A

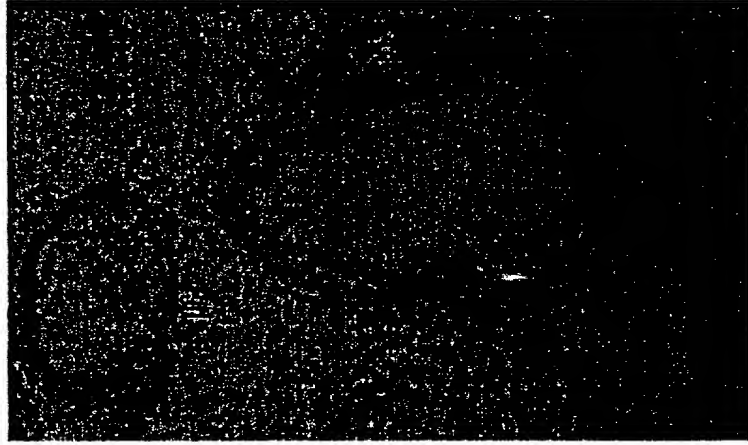


FIG. 12B

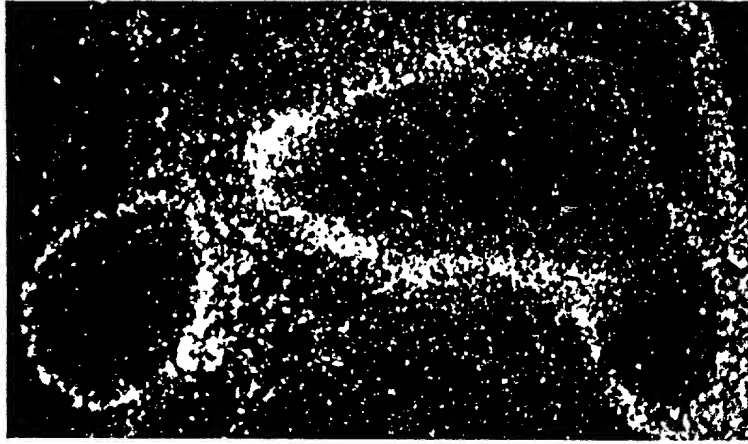


FIG. 12C

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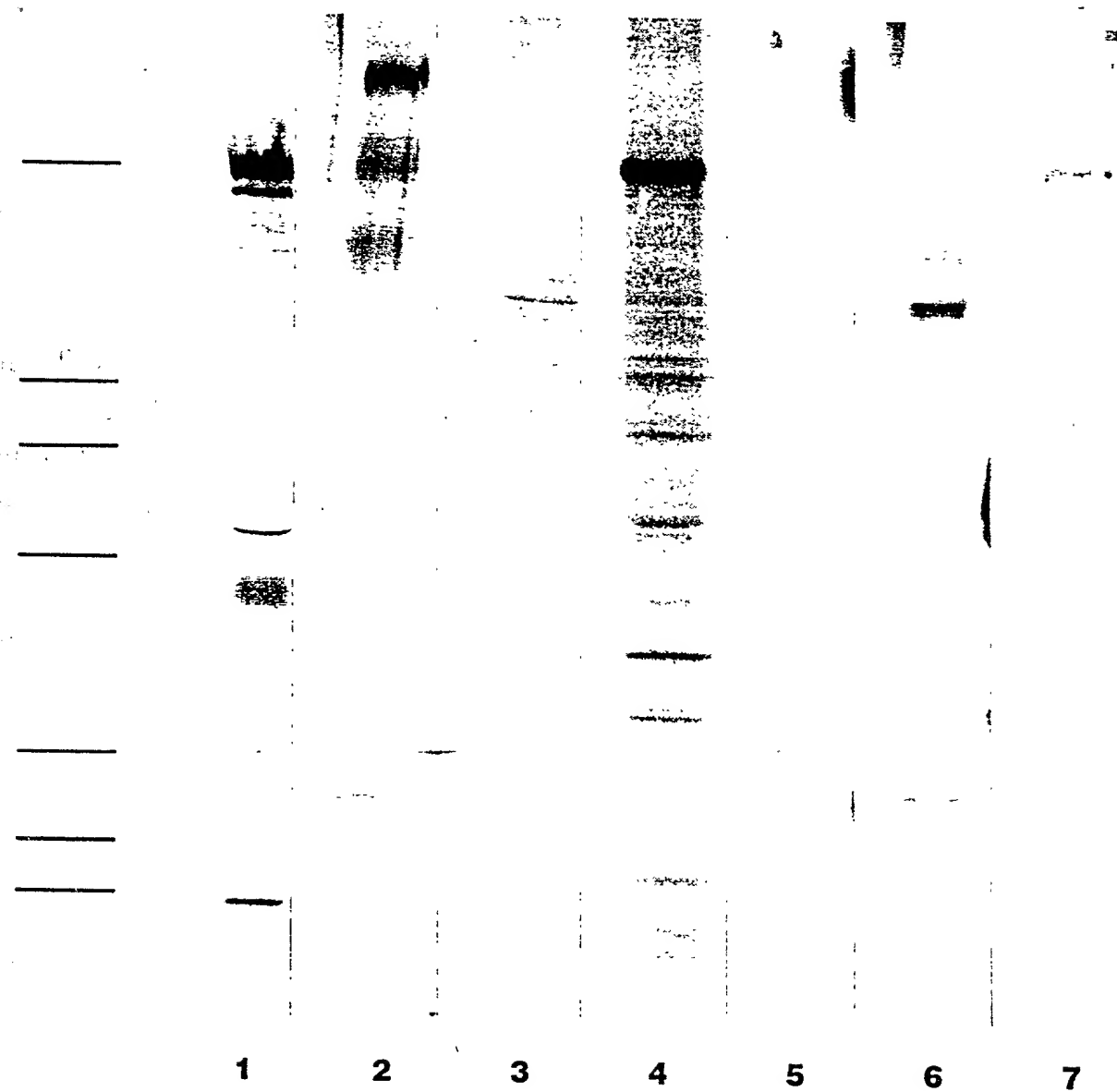


FIG. 13



PATENT
UMIC:013

DECLARATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or the below named inventors are the original, first and joint inventors (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **LATENT TGF β BINDING PROTEIN (LTBP) GENES, COMPOSITIONS AND METHODS**, the Specification of which:

is attached hereto.
X was filed on June 7, 1995 as Application Serial No. 08/479,722.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability of the subject matter claimed in this application, as "materiality" is defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

<u>PCT/US95/02251</u>	<u>February 21, 1995</u>
(Number)	(Date Filed)

Priority
Claimed

Yes/No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all information known to me to be material to patentability of the subject matter claimed in this application, as "materiality" is defined in Title 37, Code of Federal Regulations, § 1.56, which become available between the filing date of the prior application and the national or PCT international filing date of this application:

<u>08/316,650</u>	<u>September 30, 1994</u>	<u>Pending</u>
<u>08/199,780</u>	<u>February 18, 1994</u>	<u>Pending</u>
(Application Serial No.)	(Filing Date)	(Status)

I hereby direct that all correspondence and telephone calls be addressed to Shelley P.M. Fussey, Arnold, White & Durkee, P.O. Box 4433, Houston, Texas 77210 (512) 418-3000.

I hereby declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Inventor's Full Name: Jeffrey Bonadio
(First) (Initial) (Last)

Inventor's Signature: Jeffrey Bonadio

Date: 12 Sept '95 Country of Citizenship: United States

Resident Address: 1870 Briar Ridge Drive, Ann Arbor, Michigan 48108
(Include number, street name, city, state, and country)

Post Office Address: _____
(if different from residence address)

002F90"58926560

Inventor's Full Name: Wushan Yin
(First) (Initial) (Last)
Inventor's Signature: Wushan Yin
Date: Sept. 12. 95 Country of Citizenship: China
Resident Address: 1587 Beal Street, #18, Ann Arbor, Michigan 48105
(Include number, street name, city, state, and country)
Post Office Address: _____
(if different from
residence address) _____

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Jeffrey Bonadio and
Wushan Yin
Serial No.: 08/479,722
Filed: June 7, 1995
For: LATENT TGFB BINDING
PROTEIN (LTBP) GENES,
COMPOSITIONS AND METHODS

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\$
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\$
\$
\$

Group Art Unit: Unknown
Examiner: Unknown
Atty. Dkt.: UMIC:013/FUS

ELECTION UNDER 37 C.F.R. §§ 3.71
AND 3.73 AND POWER OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The undersigned, being Assignee of record of the entire interest in the above-identified application by virtue of an assignment recorded in the United States Patent and Trademark Office as set forth below, hereby elects, under 37 C.F.R. § 3.71, to prosecute the application to the exclusion of the inventors.

The Assignee hereby revokes any previous Powers of Attorney and appoints Louis T. Pirkey, Reg. No. 22,393; J. Paul Williamson, Reg. No. 29,600; David L. Parker, Reg. No. 32,165; Barbara S. Kitchell, Reg. No. 33,928; Mark B. Wilson, Reg. No. 37,259; Gary J. Sertich, Reg. No. 34,430; Steven L. Highlander, Reg. No. 37,642; Timothy S. Corder, Reg. No. 38,414; Adam V. Floyd, Reg. No. 39,192; and Shelley P.M. Fussey, Reg. No. 39,458; each an attorney or agent of the firm of ARNOLD, WHITE & DURKEE, as its attorney or agent for so long as they remain with such firm, with full power of substitution and revocation, to prosecute the application, to make alterations and amendments therein, to transact all business in the Patent and Trademark Office in connection therewith, and to receive any Letters Patent, and for one year after issuance of such Letters Patent to file any request for a certificate of correction that may be deemed appropriate.

Pursuant to 37 C.F.R. § 3.73, the undersigned has reviewed the evidentiary documents, specifically the Assignment to The Regent of the University of Michigan, referenced below, and certifies that to the best of my knowledge and belief, title remains in the name of the Assignee.

Please direct all communications as follows:

Shelley P.M. Fussey
ARNOLD, WHITE & DURKEE
P.O. Box 4433
Houston, Texas 77210-4433
(512) 418-3000

ASSIGNEE: THE REGENT OF THE
UNIVERSITY OF MICHIGAN

By: 

Name: Robert L. Robb
Title: Director of Technology
Management Office

Date: 9-13-95

ASSIGNMENT: Concurrently filed

002190 59325560

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Jeffrey Bonadio

Wushan Yin

Serial No.: 08/479,722

Filed: June 7, 1995

For: LATENT TGF β BINDING PROTEIN
(LTBP) GENES, COMPOSITIONS
AND METHODS



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Examiner: David L. Fitzgerald

Group Art Unit: 1646

Atty. Dkt: 4100.000500

Formerly: UMIC:013

ELECTION UNDER 37 C.F.R. §§ 3.71 AND 3.73
AND POWER OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The undersigned, being Assignee of record of the entire interest in the above-identified application by virtue of an assignment recorded in the United States Patent and Trademark Office as set forth below, hereby elects, under 37 C.F.R. § 3.71, to prosecute the application to the exclusion of the inventor.

The Assignee hereby revokes any previous Powers of Attorney and appoints:

Danny L. Williams, Reg. No. 31,892; Terry D. Morgan, Reg. No. 31,181; J. Mike Amerson, Reg. No. 35,426; Kenneth D. Goodman, Reg. No. 30,460; Barbara S. Kitchell, Reg. No. 33,928; Jeffrey A. Pyle, Reg. No. 34,904; Randall C. Furlong, Reg. No. 35,144; Scott F. Diring, Reg. No. 35,119; Jeffrey E. Hundley, Reg. No. 42,676; George J. Oehling, Reg. No. 40,471; Shelley P.M. Fussey, Reg. No. 39,458; David W. Hibler, Reg. No. 41,071; and Mark D. Moore, Reg. No. 42,903.

each an attorney or agent with the law firm of WILLIAMS, MORGAN & AMERSON, P.C., as its attorney or agent so long as they remain with such law firm, with full power of substitution and revocation, to prosecute the application, to make alterations and amendments therein, to transact all business in the Patent and Trademark Office in connection therewith, and to receive any Letters Patent, and for one year after issuance of such Letters Patent to file any request for a certificate of correction that may be deemed appropriate.

Pursuant to 37 C.F.R. § 3.73, the undersigned has reviewed the evidentiary documents, specifically the Assignment to **The Regents of The University of Michigan**, referenced below, and certifies that to the best of my knowledge and behalf, title remains in the name of the Assignee.

Please direct all communications as follows:

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ASSIGNEE:
THE REGENTS OF THE
UNIVERSITY OF MICHIGAN

BY: Marvin G. Parnes
Name: Marvin G. Parnes
Title: Associate VP for Research
Interim Director of Technology
Management Office

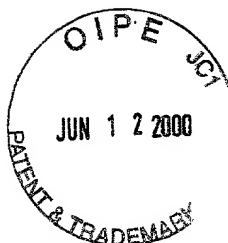
Date: 3-31-99

ASSIGNMENT:

☐
☒

Concurrently filed
Previously recorded

Date: 09/18/1995
Reel: 7650
Frame: 0299



SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Bonadio, Jeffrey
Yin, Wushan
- (ii) TITLE OF INVENTION: LATENT TGF(BINDING PROTEIN (LTBP)
GENES, COMPOSITIONS AND METHODS
- (iii) NUMBER OF SEQUENCES: 13
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Williams, Morgan & Amerson
 - (B) STREET: 7676 Hillmont, Suite 250
 - (C) CITY: Houston
 - (D) STATE: Texas
 - (E) COUNTRY: USA
 - (F) ZIP: 77040
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) -OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/479,722
 - (B) FILING DATE: 07-JUN-1995
 - (C) CLASSIFICATION: Unknown
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US PCT/US95/02251
 - (B) FILING DATE: 21-FEB-1995
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/316,650
 - (B) FILING DATE: 30-SEP-1994
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/199,780
 - (B) FILING DATE: 18-FEB-1994
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Fussey, Shelley P.M.
 - (B) REGISTRATION NUMBER: 39,458
 - (C) REFERENCE/DOCKET NUMBER: 4100.000500/FUS
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (713) 934-7000
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5499 base pairs

(B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(A) DESCRIPTION: /desc = "DNA"

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..5499

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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His Ser Gly Ala Met Arg Ala Pro Thr Thr Ala Arg Cys Ser Gly Cys	
20 25 30	
ATC CAA CGG GTG CGT TGG AGG GGC TTC CTG CCA CTT GTC CTG GCT GTC	144
Ile Gln Arg Val Arg Trp Arg Gly Phe Leu Pro Leu Val Leu Ala Val	
35 40 45	
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Leu Met Gly Thr Ser His Ala Gln Arg Asp Ser Ile Gly Arg Tyr Glu	
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Pro Ala Ser Arg Asp Ala Asn Arg Leu Trp His Pro Val Gly Ser His	
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CCC GCA GCG GCT GCA GCC AAG GTG TAC AGT CTG TTC CGA GAG CCT GAC	288
Pro Ala Ala Ala Ala Lys Val Tyr Ser Leu Phe Arg Glu Pro Asp	
85 90 95	
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Ala Pro Val Pro Gly Leu Ser Pro Ser Glu Trp Asn Gln Pro Ala Gln	
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GGG AAC CCG GGA TGG CTC GCA GAG GCC GAG GCC AGG AGG CCA CCT CGA	384
Gly Asn Pro Gly Trp Leu Ala Glu Ala Glu Ala Arg Arg Pro Pro Arg	
115 120 125	
ACC CAG CAG CTG CGT CGA GTC CAG CCA CCT GTC CAG ACT CGG AGA AGC	432
Thr Gln Gln Leu Arg Arg Val Gln Pro Pro Val Gln Thr Arg Arg Ser	
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CAT CCC CGG GGC CAG CAG CAG ATA GCA GCC CGG GCT GCA CCT TCT GTC	480
His Pro Arg Gly Gln Gln Gln Ile Ala Ala Arg Ala Ala Pro Ser Val	
145 150 155 160	
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Ala Arg Leu Glu Thr Pro Gln Arg Pro Ala Ala Ala Arg Arg Gly Arg	
165 170 175	
CTC ACT GGG AGA AAT GTC TGC GGG GGA CAG TGC TGC CCA GGA TGG ACA	576

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Leu Thr Gly Arg Asn Val Cys Gly Gly Gln Cys Cys Pro Gly Trp Thr	
180 185 190	
ACA TCA AAC AGC ACC AAC CAC TGT ATC AAA CCT GTG TGT CAG CCT CCC	624
Thr Ser Asn Ser Thr Asn His Cys Ile Lys Pro Val Cys Gln Pro Pro	
195 200 205	
TGT CAG AAC CGA GGC TCC TGC AGC AGG CCC CAG GTC TGC ATC TGC CGT	672
Cys Gln Asn Arg Gly Ser Cys Ser Arg Pro Gln Val Cys Ile Cys Arg	
210 215 220	
TCT GGC TTC CGT GGG GCG CGC TGT GAG GAG GTC ATC CCT GAG GAG GAA	720
Ser Gly Phe Arg Gly Ala Arg Cys Glu Glu Val Ile Pro Glu Glu Glu	
225 230 235 240	
TTT GAC CCT CAG AAT GCC AGG CCT GTG CCC AGA CGC TCA GTG GAG AGA	768
Phe Asp Pro Gln Asn Ala Arg Pro Val Pro Arg Arg Ser Val Glu Arg	
245 250 255	
GCA CCC GGT CCT CAC AGA AGC AGT GAG GCC AGA GGA AGT CTA GTG ACC	816
Ala Pro Gly Pro His Arg Ser Ser Glu Ala Arg Gly Ser Leu Val Thr	
260 265 270	
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Arg Ile Gln Pro Leu Val Pro Pro Pro Ser Pro Pro Pro Ser Arg Arg	
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GTT CGT CGG TAT CCG GCC ACT GGT GCC AAT GGC CAG CTG ATG TCC AAC	960
Val Arg Arg Tyr Pro Ala Thr Gly Ala Asn Gly Gln Leu Met Ser Asn	
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Ala Leu Pro Ser Gly Leu Glu Leu Arg Asp Ser Ser Pro Gln Ala Ala	
325 330 335	
CAT GTG AAC CAT CTC TCA CCC CCC TGG GGG CTG AAC CTC ACC GAG AAA	1056
His Val Asn His Leu Ser Pro Pro Trp Gly Leu Asn Leu Thr Glu Lys	
340 345 350	
ATC AAG AAA ATC AAA GTC GTC TTC ACC CCC ACC ATC TGC AAG CAG ACC	1104
Ile Lys Lys Ile Lys Val Val Phe Thr Pro Thr Ile Cys Lys Gln Thr	
355 360 365	
TGT GCC CGG GGA CGC TGT GCC AAC AGC TGT GAG AAG GGT GAC ACC ACC	1152
Cys Ala Arg Gly Arg Cys Ala Asn Ser Cys Glu Lys Gly Asp Thr Thr	
370 375 380	
ACC TTG TAC AGT CAG GGT GGC CAT GGG CAT GAC CCC AAG TCT GGC TTC	1200
Thr Leu Tyr Ser Gln Gly Gly His Gly His Asp Pro Lys Ser Gly Phe	
385 390 395 400	
CGT ATC TAT TTC TGC CAA ATC CCC TGC CTG AAT GGT GGC CGC TGC ATC	1248
Arg Ile Tyr Phe Cys Gln Ile Pro Cys Leu Asn Gly Gly Arg Cys Ile	

405	410	415	
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CAT CTG CCT GTC CCG CAG CCA GAC AGG GAA CCT GCA GGG CGA GGT TCC His Leu Pro Val Pro Gln Pro Asp Arg Glu Pro Ala Gly Arg Gly Ser 435 440 445			1344
CGG CAC AGA ACC CTG CTG GAA GGT CCC CTG AAG CAA TCC ACC TTC ACG Arg His Arg Thr Leu Leu Glu Gly Pro Leu Lys Gln Ser Thr Phe Thr 450 455 460			1392
CTG CCT CTC TCT AAC CAG CTC GCC TCT GTG AAC CCC TCG CTG GTG AAG Leu Pro Leu Ser Asn Gln Leu Ala Ser Val Asn Pro Ser Leu Val Lys 465 470 475 480			1440
GTG CAA ATT CAT CAC CCG CCT GAG GCC TCT GTG CAG ATT CAC CAG GTG Val Gln Ile His His Pro Pro Glu Ala Ser Val Gln Ile His Gln Val 485 490 495			1488
GCC CGG GTC CGG GGT GAG CTG GAC CCC GTG CTG GAG GAC AAC AGT GTG Ala Arg Val Arg Gly Glu Leu Asp Pro Val Leu Glu Asp Asn Ser Val 500 505 510			1536
GAG ACC AGA GCC TCT CAT CGC CCC CAC GGC AAC CTA GGC CAC AGC CCC Glu Thr Arg Ala Ser His Arg Pro His Gly Asn Leu Gly His Ser Pro 515 520 525			1584
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CCT GGC CTC ATG CTG GAT CCG TCA AGG AGC CGC TGC GTA TCG GAC AAG Pro Gly Leu Met Leu Asp Pro Ser Arg Ser Arg Cys Val Ser Asp Lys 660 665 670	2016
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ACC TGC ACC CTG CCT TTG GTT CAT CGG ATC ACC AAG CAG ATA TGC TGC Thr Cys Thr Leu Pro Leu Val His Arg Ile Thr Lys Gln Ile Cys Cys 690 695 700	2112
TGC AGC CGT GTG GGC AAA GCC TGG GGT AGC ACA TGT GAA CAG TGT CCC Cys Ser Arg Val Gly Lys Ala Trp Gly Ser Thr Cys Glu Gln Cys Pro 705 710 715 720	2160
CTG CCT GGC ACA GAA GCC TTC AGG GAG ATC TGC CCT GCT GGC CAT GGC Leu Pro Gly Thr Glu Ala Phe Arg Glu Ile Cys Pro Ala Gly His Gly 725 730 735	2208
TAC ACC TAC TCG AGC TCA GAC ATC CGC CTG TCT ATG AGG AAA GCC GAA Tyr Thr Tyr Ser Ser Ser Asp Ile Arg Leu Ser Met Arg Lys Ala Glu 740 745 750	2256
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GCA CCC CCA CCT GGG CAA GCA GAG AGG CAA CCA CTC CGG GCA GCC ACC Ala Pro Pro Pro Gly Gln Ala Glu Arg Gln Pro Leu Arg Ala Ala Thr 770 775 780	2352
GCC ACC TGG ATT GAG GCT GAG ACC CTC CCT GAC AAA GGT GAC TCT CGG Ala Thr Trp Ile Glu Ala Glu Thr Leu Pro Asp Lys Gly Asp Ser Arg 785 790 795 800	2400
GCT GTT CAG ATC ACA ACC AGT GCT CCC CAC CTA CCT GCC CGG GTA CCA Ala Val Gln Ile Thr Thr Ser Ala Pro His Leu Pro Ala Arg Val Pro 805 810 815	2448
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CCA GAG AGT CCA GCA GAA GAG CAA GTG ATT CCC TCC AGT GAT GTC TTG Pro Glu Ser Pro Ala Glu Glu Gln Val Ile Pro Ser Ser Asp Val Leu 835 840 845	2544
GTG ACA CAC AGC CCC CCA GAC TTT GAT CCA TGT TTT GCT GGA GCC TCC Val Thr His Ser Pro Pro Asp Phe Asp Pro Cys Phe Ala Gly Ala Ser 850 855 860	2592

AAC ATC TGT GGC CCT GGG ACC TGT GTG AGC CTC CCA AAT GGA TAC AGA Asn Ile Cys Gly Pro Gly Thr Cys Val Ser Leu Pro Asn Gly Tyr Arg 865 870 875 880	2640
TGT GTC TGC AGC CCT GGC TAC CAG CTA CAC CCC AGC CAA GAC TAC TGT Cys Val Cys Ser Pro Gly Tyr Gln Leu His Pro Ser Gln Asp Tyr Cys 885 890 895	2688
ACT GAT GAC AAC GAG TGT ATG AGG AAC CCC TGT GAA GGA AGA GGG CGC Thr Asp Asp Asn Glu Cys Met Arg Asn Pro Cys Glu Gly Arg Gly Arg 900 905 910	2736
TGT GTC AAC AGT GTG GGC TCC TAC TCC TGC CTC TGC TAT CCT GGC TAC Cys Val Asn Ser Val Gly Ser Tyr Ser Cys Leu Cys Tyr Pro Gly Tyr 915 920 925	2784
ACA CTA GTC ACC CTC GGA GAC ACA CAG GAG TGC CAA GAT ATC GAT GAG Thr Leu Val Thr Leu Gly Asp Thr Gln Glu Cys Gln Asp Ile Asp Glu 930 935 940	2832
TGT GAG CAG CCC GGG GTG TGC AGT GGT GGG CGA TGC AGC AAC ACG GAG Cys Glu Gln Pro Gly Val Cys Ser Gly Gly Arg Cys Ser Asn Thr Glu 945 950 955 960	2880
GGC TCG TAC CAC TGC GAG TGT GAT CGG GGC TAC ATC ATG GTC AGG AAA Gly Ser Tyr His Cys Glu Cys Asp Arg Gly Tyr Ile Met Val Arg Lys 965 970 975	2928
GGA CAC TGT CAA GAT ATC AAC GAA TGC CGT CAC CCT GGT ACC TGC CCT Gly His Cys Gln Asp Ile Asn Glu Cys Arg His Pro Gly Thr Cys Pro 980 985 990	2976
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GAG GAG GGC TAT GTA GGC CAG AGT GGG AGC TGT GTA GAT GTC AAT GAG Glu Glu Gly Tyr Val Gly Gln Ser Gly Ser Cys Val Asp Val Asn Glu 1010 1015 1020	3072
TGT CTG ACC CCT GGG ATA TGT ACC CAT GGA AGG TGC ATC AAC ATG GAA Cys Leu Thr Pro Gly Ile Cys Thr His Gly Arg Cys Ile Asn Met Glu 1025 1030 1035 1040	3120
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CCC ACG GGC CTC TGC CTC AAC ACG GAG GGC TCC TTC ACC TGC TCA GCC Pro Thr Gly Leu Cys Leu Asn Thr Glu Gly Ser Phe Thr Cys Ser Ala 1075 1080 1085	3264
TGT CAG AGC GGG TAC TGG GTG AAC GAA GAT GGC ACT GCC TGT GAA GAC	3312

Cys Gln Ser Gly Tyr Trp Val Asn Glu Asp Gly Thr Ala Cys Glu Asp	
1090	1095 1100
TTG GAT GAA TGT GCC TTC CCT GGA GTC TGC CCC ACA GGC GTC TGC ACC	3360
Leu Asp Glu Cys Ala Phe Pro Gly Val Cys Pro Thr Gly Val Cys Thr	
1105	1110 1115 1120
AAT ACT GTA GGC TCC TTC TCC TGC AAG GAC TGT GAC CAG GGC TAC CGG	3408
Asn Thr Val Gly Ser Phe Ser Cys Lys Asp Cys Asp Gln Gly Tyr Arg	
	1125 1130 1135
CCC AAC CCC CTG GGC AAC AGA TGC GAA GAT GTG GAT GAG TGT GAA GGT	3456
Pro Asn Pro Leu Gly Asn Arg Cys Glu Asp Val Asp Glu Cys Glu Gly	
	1140 1145 1150
CCC CAA AGC AGC TGC CGG GGA GGC GAA TGC AAG AAC ACA GAA GGT TCC	3504
Pro Gln Ser Ser Cys Arg Gly Gly Glu Cys Lys Asn Thr Glu Gly Ser	
	1155 1160 1165
TAC CAA TGC CTC TGT CAC CAG GGC TTC CAG CTG GTC AAT GGC ACC ATG	3552
Tyr Gln Cys Leu Cys His Gln Gly Phe Gln Leu Val Asn Gly Thr Met	
	1170 1175 1180
TGT GAG GAC GTG AAT GAG TGT GTT GGG GAA GAG CAT TGT GCT CCT CAC	3600
Cys Glu Asp Val Asn Glu Cys Val Gly Glu Glu His Cys Ala Pro His	
	1185 1190 1195 1200
GGC GAG TGC CTC AAC AGC CTG GGC TCC TTC TTC TGC CTC TGT GCA CCC	3648
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GGC TTT GCT AGT GCT GAG GGG GGC ACC AGA TGC CAG GAT GTT GAT GAA	3696
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	1220 1225 1230
TGT GCA GCC ACA GAC CCG TGT CCG GGA GGA CAC TGT GTC AAC ACA GAG	3744
Cys Ala Ala Thr Asp Pro Cys Pro Gly Gly His Cys Val Asn Thr Glu	
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GGC TCC TTC AGC TGT CTG TGT GAG ACT GCT TCC TTC CAG CCC TCC CCA	3792
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	1250 1255 1260
GAC AGC GGA GAA TGT TTG GAT ATT GAT GAG TGT GAG GAC CGT GAA GAC	3840
Asp Ser Gly Glu Cys Leu Asp Ile Asp Glu Cys Glu Asp Arg Glu Asp	
	1265 1270 1275 1280
CCG GTG TGC GGA GCC TGG AGG TGT GAG AAC AGT CCT GGT TCC TAC CGC	3888
Pro Val Cys Gly Ala Trp Arg Cys Glu Asn Ser Pro Gly Ser Tyr Arg	
	1285 1290 1295
TGC ATC CTG GAC TGC CAG CCT GGA TTC TAT GTG GCG CCA AAT GGA GAC	3936
Cys Ile Leu Asp Cys Gln Pro Gly Phe Tyr Val Ala Pro Asn Gly Asp	
	1300 1305 1310
TGC ATT GAC ATA GAT GAA TGT GCC AAT GAC ACT GTG TGT GGG AAC CAT	3984
Cys Ile Asp Ile Asp Glu Cys Ala Asn Asp Thr Val Cys Gly Asn His	

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GGC TTC GAG ACC TCA CCA TCA GGC TGG GAG TGT GTT GAT GTG AAC GAG Gly Phe Glu Thr Ser Pro Ser Gly Trp Glu Cys Val Asp Val Asn Glu 1345 1350 1355 1360			4080
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GAA GGC TCC TTC CTG TGC CTT TGC GCC AGT GAC CTT GAG GAG TAC GAC Glu Gly Ser Phe Leu Cys Leu Cys Ala Ser Asp Leu Glu Glu Tyr Asp 1380 1385 1390			4176
GCA GAA GAA GGA CAC TGC CGT CCT CGG GTG GCT GGA GCT CAG AGA ATC Ala Glu Glu Gly His Cys Arg Pro Arg Val Ala Gly Ala Gln Arg Ile 1395 1400 1405			4224
CCA GAG GTC CGG ACA GAG GAC CAG GCT CCA AGC CTT ATC CGC ATG GAA Pro Glu Val Arg Thr Glu Asp Gln Ala Pro Ser Leu Ile Arg Met Glu 1410 1415 1420			4272
TGC TAC TCT GAA CAC AAT GGT GGT CCT CCC TGC TCT CAA ATC CTG GGC Cys Tyr Ser Glu His Asn Gly Gly Pro Pro Cys Ser Gln Ile Leu Gly 1425 1430 1435 1440			4320
CAG AAC TCC ACA CAG GCC GAG TGC TGC TGC ACT CAG GGT GCC AGA TGG Gln Asn Ser Thr Gln Ala Glu Cys Cys Cys Thr Gln Gly Ala Arg Trp 1445 1450 1455			4368
GGA AAG GCC TGT GCG CCC TGC CCA TCT GAG GAC TCA GTT GAA TTC AGT Gly Lys Ala Cys Ala Pro Cys Pro Ser Glu Asp Ser Val Glu Phe Ser 1460 1465 1470			4416
CAG CTC TGC CCC AGT GGT CAA GGT TAC ATC CCA GTG GAA GGA GCC TGG Gln Leu Cys Pro Ser Gly Gln Gly Tyr Ile Pro Val Glu Gly Ala Trp 1475 1480 1485			4464
ACA TTT GGA CAA ACC ATG TAT ACA GAT GCC GAT GAA TGT GTA CTG TTT Thr Phe Gly Gln Thr Met Tyr Thr Asp Ala Asp Glu Cys Val Leu Phe 1490 1495 1500			4512
GGG CCT GCT CTC TGC CAG AAT GGC CGA TGC TCA AAC ATA GTG CCT GGC Gly Pro Ala Leu Cys Gln Asn Gly Arg Cys Ser Asn Ile Val Pro Gly 1505 1510 1515 1520			4560
TAC ATT TGC CTG TGC AAC CCT GGC TAC CAC TAT GAT GCC TCC AGC AGG Tyr Ile Cys Leu Cys Asn Pro Gly Tyr His Tyr Asp Ala Ser Ser Arg 1525 1530 1535			4608
AAG TGC CAG GAT CAC AAC GAA TGC CAG GAC TTG GCC TGT GAG AAC GGT Lys Cys Gln Asp His Asn Glu Cys Gln Asp Leu Ala Cys Glu Asn Gly 1540 1545 1550			4656

GAG TGT GTG AAC CAA GAA GGC TCC TTC CAT TGC CTC TGC AAT CCC CCC Glu Cys Val Asn Gln Glu Gly Ser Phe His Cys Leu Cys Asn Pro Pro 1555 1560 1565	4704
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ACG GAG GAC TTC CCT GAC CAT GAC ATC CAC ATG GAC ATC TGC TGG AAA Thr Glu Asp Phe Pro Asp His Asp Ile His Met Asp Ile Cys Trp Lys 1585 1590 1595 1600	4800
AAA GTC ACC AAT GAT GTG TGC AGC CAG CCC TTG CGT GGG CAC CAT ACC Lys Val Thr Asn Asp Val Cys Ser Gln Pro Leu Arg Gly His His Thr 1605 1610 1615	4848
ACC TAT ACA GAA TGC TGC TGC CAA GAT GGG GAG GCC TGG AGC CAG CAA Thr Tyr Thr Glu Cys Cys Cys Gln Asp Gly Glu Ala Trp Ser Gln Gln 1620 1625 1630	4896
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AAC GTG GCT CGG ATT GAG GCA GAG CGC GGA GCA GGG ATC CAC TTC CGG Asn Val Ala Arg Ile Glu Ala Glu Arg Gly Ala Gly Ile His Phe Arg 1650 1655 1660	4992
CCA GGC TAT GAG TAT GGC CCT GGC CTG GAC GAT CTG CCT GAA AAC CTC Pro Gly Tyr Glu Tyr Gly Pro Gly Leu Asp Asp Leu Pro Glu Asn Leu 1665 1670 1675 1680	5040
TAC GGC CCA GAT GGG GCT CCC TTC TAT AAC TAC CTA GGC CCC GAG GAC Tyr Gly Pro Asp Gly Ala Pro Phe Tyr Asn Tyr Leu Gly Pro Glu Asp 1685 1690 1695	5088
ACT GCC CCT GAG CCT CCC TTC TCC AAC CCA GCC AGC CAG CCG GGA GAC Thr Ala Pro Glu Pro Pro Phe Ser Asn Pro Ala Ser Gln Pro Gly Asp 1700 1705 1710	5136
AAC ACA CCT GTC CTT GAG CCT CCT CTG CAG CCC TCT GAA CTT CAG CCT Asn Thr Pro Val Leu Glu Pro Pro Leu Gln Pro Ser Glu Leu Gln Pro 1715 1720 1725	5184
CAC TAT CTA GCC AGC CAC TCA GAA CCC CCT GCC TCC TTC GAA GGC CTT His Tyr Leu Ala Ser His Ser Glu Pro Pro Ala Ser Phe Glu Gly Leu 1730 1735 1740	5232
CAG GCT GAG GAA TGT GGC ATC CTG AAT GGC TGT GAG AAT GGC CGC TGC Gln Ala Glu Glu Cys Gly Ile Leu Asn Gly Cys Glu Asn Gly Arg Cys 1745 1750 1755 1760	5280
GTG CGT GTG CGG GAG GGC TAC ACT TGC GAC TGC TTT GAG GGC TTC CAG Val Arg Val Arg Glu Gly Tyr Thr Cys Asp Cys Phe Glu Gly Phe Gln 1765 1770 1775	5328

CTG GAT GCG CCC ACA TTG GCC TGT GTG GAT GTG AAC GAG TGT GAA GAC 5376
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 1780 1785 1790

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 1795 1800 1805

GAG GGT TCC TAT CGC TGC CAC TGT TCG CCA GGT TAC GTG GCA GAG CCA 5472
 Glu Gly Ser Thr Arg Cys His Cys Ser Pro Gly Tyr Val Ala Glu Pro
 1810 1815 1820

GGC CCC CCA CAC TGT GCG GCC AAG GAG 5499
 Gly Pro Pro His Cys Ala Ala Lys Glu
 1825 1830

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1833 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Ser Thr Ser Pro Arg Gly Leu Arg Cys Pro Gln Leu Cys Ser
 1 5 10 15
 His Ser Gly Ala Met Arg Ala Pro Thr Thr Ala Arg Cys Ser Gly Cys
 20 25 30
 Ile Gln Arg Val Arg Trp Arg Gly Phe Leu Pro Leu Val Leu Ala Val
 35 40 45
 Leu Met Gly Thr Ser His Ala Gln Arg Asp Ser Ile Gly Arg Tyr Glu
 50 55 60
 Pro Ala Ser Arg Asp Ala Asn Arg Leu Trp His Pro Val Gly Ser His
 65 70 75 80
 Pro Ala Ala Ala Ala Ala Lys Val Tyr Ser Leu Phe Arg Glu Pro Asp
 85 90 95
 Ala Pro Val Pro Gly Leu Ser Pro Ser Glu Trp Asn Gln Pro Ala Gln
 100 105 110
 Gly Asn Pro Gly Trp Leu Ala Glu Ala Glu Ala Arg Arg Pro Pro Arg
 115 120 125
 Thr Gln Gln Leu Arg Arg Val Gln Pro Pro Val Gln Thr Arg Arg Ser
 130 135 140
 His Pro Arg Gly Gln Gln Gln Ile Ala Ala Arg Ala Ala Pro Ser Val
 145 150 155 160

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Ala	Arg	Leu	Glu	Thr	Pro	Gln	Arg	Pro	Ala	Ala	Ala	Arg	Arg	Gly	Arg		165	170	175	
Leu	Thr	Gly	Arg	Asn	Val	Cys	Gly	Gly	Gln	Cys	Cys	Pro	Gly	Trp	Thr		180	185	190	
Thr	Ser	Asn	Ser	Thr	Asn	His	Cys	Ile	Lys	Pro	Val	Cys	Gln	Pro	Pro		195	200	205	
Cys	Gln	Asn	Arg	Gly	Ser	Cys	Ser	Arg	Pro	Gln	Val	Cys	Ile	Cys	Arg		210	215	220	
Ser	Gly	Phe	Arg	Gly	Ala	Arg	Cys	Glu	Glu	Val	Ile	Pro	Glu	Glu	Glu		225	230	235	240
Phe	Asp	Pro	Gln	Asn	Ala	Arg	Pro	Val	Pro	Arg	Arg	Ser	Val	Glu	Arg		245	250	255	
Ala	Pro	Gly	Pro	His	Arg	Ser	Ser	Glu	Ala	Arg	Gly	Ser	Leu	Val	Thr		260	265	270	
Arg	Ile	Gln	Pro	Leu	Val	Pro	Pro	Pro	Ser	Pro	Pro	Pro	Ser	Arg	Arg		275	280	285	
Leu	Ser	Gln	Pro	Trp	Pro	Leu	Gln	Gln	His	Ser	Gly	Pro	Ser	Arg	Thr		290	295	300	
Val	Arg	Arg	Tyr	Pro	Ala	Thr	Gly	Ala	Asn	Gly	Gln	Leu	Met	Ser	Asn		305	310	315	320
Ala	Leu	Pro	Ser	Gly	Leu	Glu	Leu	Arg	Asp	Ser	Ser	Pro	Gln	Ala	Ala		325	330	335	
His	Val	Asn	His	Leu	Ser	Pro	Pro	Trp	Gly	Leu	Asn	Leu	Thr	Glu	Lys		340	345	350	
Ile	Lys	Lys	Ile	Lys	Val	Val	Phe	Thr	Pro	Thr	Ile	Cys	Lys	Gln	Thr		355	360	365	
Cys	Ala	Arg	Gly	Arg	Cys	Ala	Asn	Ser	Cys	Glu	Lys	Gly	Asp	Thr	Thr		370	375	380	
Thr	Leu	Tyr	Ser	Gln	Gly	Gly	His	Gly	His	Asp	Pro	Lys	Ser	Gly	Phe		385	390	395	400
Arg	Ile	Tyr	Phe	Cys	Gln	Ile	Pro	Cys	Leu	Asn	Gly	Gly	Arg	Cys	Ile		405	410	415	
Gly	Arg	Asp	Glu	Cys	Trp	Cys	Pro	Ala	Asn	Ser	Thr	Gly	Lys	Phe	Cys		420	425	430	
His	Leu	Pro	Val	Pro	Gln	Pro	Asp	Arg	Glu	Pro	Ala	Gly	Arg	Gly	Ser		435	440	445	
Arg	His	Arg	Thr	Leu	Leu	Glu	Gly	Pro	Leu	Lys	Gln	Ser	Thr	Phe	Thr		450	455	460	

Ala Pro Pro Pro Gly Gln Ala Glu Arg Gln Pro Leu Arg Ala Ala Thr
 770 775 780
 Ala Thr Trp Ile Glu Ala Glu Thr Leu Pro Asp Lys Gly Asp Ser Arg
 785 790 795 800
 Ala Val Gln Ile Thr Thr Ser Ala Pro His Leu Pro Ala Arg Val Pro
 805 810 815
 Gly Asp Ala Thr Gly Arg Pro Ala Pro Ser Leu Pro Gly Gln Gly Ile
 820 825 830
 Pro Glu Ser Pro Ala Glu Glu Gln Val Ile Pro Ser Ser Asp Val Leu
 835 840 845
 Val Thr His Ser Pro Pro Asp Phe Asp Pro Cys Phe Ala Gly Ala Ser
 850 855 860
 Asn Ile Cys Gly Pro Gly Thr Cys Val Ser Leu Pro Asn Gly Tyr Arg
 865 870 875 880
 Cys Val Cys Ser Pro Gly Tyr Gln Leu His Pro Ser Gln Asp Tyr Cys
 885 890 895
 Thr Asp Asp Asn Glu Cys Met Arg Asn Pro Cys Glu Gly Arg Gly Arg
 900 905 910
 Cys Val Asn Ser Val Gly Ser Tyr Ser Cys Leu Cys Tyr Pro Gly Tyr
 915 920 925
 Thr Leu Val Thr Leu Gly Asp Thr Gln Glu Cys Gln Asp Ile Asp Glu
 930 935 940
 Cys Glu Gln Pro Gly Val Cys Ser Gly Gly Arg Cys Ser Asn Thr Glu
 945 950 955 960
 Gly Ser Tyr His Cys Glu Cys Asp Arg Gly Tyr Ile Met Val Arg Lys
 965 970 975
 Gly His Cys Gln Asp Ile Asn Glu Cys Arg His Pro Gly Thr Cys Pro
 980 985 990
 Asp Gly Arg Cys Val Asn Ser Pro Gly Ser Tyr Thr Cys Leu Ala Cys
 995 1000 1005
 Glu Glu Gly Tyr Val Gly Gln Ser Gly Ser Cys Val Asp Val Asn Glu
 1010 1015 1020
 Cys Leu Thr Pro Gly Ile Cys Thr His Gly Arg Cys Ile Asn Met Glu
 1025 1030 1035 1040
 Gly Ser Phe Arg Cys Ser Cys Glu Pro Gly Tyr Glu Val Thr Pro Asp
 1045 1050 1055
 Lys Lys Gly Cys Arg Asp Val Asp Glu Cys Ala Ser Arg Ala Ser Cys
 1060 1065 1070

Glu Gly Ser Phe Leu Cys Leu Cys Ala Ser Asp Leu Glu Glu Tyr Asp	1380	1385	1390
Ala Glu Glu Gly His Cys Arg Pro Arg Val Ala Gly Ala Gln Arg Ile	1395	1400	1405
Pro Glu Val Arg Thr Glu Asp Gln Ala Pro Ser Leu Ile Arg Met Glu	1410	1415	1420
Cys Tyr Ser Glu His Asn Gly Gly Pro Pro Cys Ser Gln Ile Leu Gly	1425	1430	1435
Gln Asn Ser Thr Gln Ala Glu Cys Cys Cys Thr Gln Gly Ala Arg Trp	1445	1450	1455
Gly Lys Ala Cys Ala Pro Cys Pro Ser Glu Asp Ser Val Glu Phe Ser	1460	1465	1470
Gln Leu Cys Pro Ser Gly Gln Gly Tyr Ile Pro Val Glu Gly Ala Trp	1475	1480	1485
Thr Phe Gly Gln Thr Met Tyr Thr Asp Ala Asp Glu Cys Val Leu Phe	1490	1495	1500
Gly Pro Ala Leu Cys Gln Asn Gly Arg Cys Ser Asn Ile Val Pro Gly	1505	1510	1515
Tyr Ile Cys Leu Cys Asn Pro Gly Tyr His Tyr Asp Ala Ser Ser Arg	1525	1530	1535
Lys Cys Gln Asp His Asn Glu Cys Gln Asp Leu Ala Cys Glu Asn Gly	1540	1545	1550
Glu Cys Val Asn Gln Glu Gly Ser Phe His Cys Leu Cys Asn Pro Pro	1555	1560	1565
Leu Thr Leu Asp Leu Ser Gly Gln Arg Cys Val Asn Thr Thr Ser Ser	1570	1575	1580
Thr Glu Asp Phe Pro Asp His Asp Ile His Met Asp Ile Cys Trp Lys	1585	1590	1595
Lys Val Thr Asn Asp Val Cys Ser Gln Pro Leu Arg Gly His His Thr	1605	1610	1615
Thr Tyr Thr Glu Cys Cys Cys Gln Asp Gly Glu Ala Trp Ser Gln Gln	1620	1625	1630
Cys Ala Leu Cys Pro Pro Arg Ser Ser Glu Val Tyr Ala Gln Leu Cys	1635	1640	1645
Asn Val Ala Arg Ile Glu Ala Glu Arg Gly Ala Gly Ile His Phe Arg	1650	1655	1660
Pro Gly Tyr Glu Tyr Gly Pro Gly Leu Asp Asp Leu Pro Glu Asn Leu	1665	1670	1675
			1680

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Tyr Gly Pro Asp Gly Ala Pro Phe Tyr Asn Tyr Leu Gly Pro Glu Asp
1685 1690 1695

Thr Ala Pro Glu Pro Pro Phe Ser Asn Pro Ala Ser Gln Pro Gly Asp
1700 1705 1710

Asn Thr Pro Val Leu Glu Pro Pro Leu Gln Pro Ser Glu Leu Gln Pro
1715 1720 1725

His Tyr Leu Ala Ser His Ser Glu Pro Pro Ala Ser Phe Glu Gly Leu
1730 1735 1740

Gln Ala Glu Glu Cys Gly Ile Leu Asn Gly Cys Glu Asn Gly Arg Cys
1745 1750 1755 1760

Val Arg Val Arg Glu Gly Tyr Thr Cys Asp Cys Phe Glu Gly Phe Gln
1765 1770 1775

Leu Asp Ala Pro Thr Leu Ala Cys Val Asp Val Asn Glu Cys Glu Asp
1780 1785 1790

Leu Asn Gly Pro Ala Arg Leu Cys Ala His Gly His Cys Glu Asn Thr
1795 1800 1805

Glu Gly Ser Tyr Arg Cys His Cys Ser Pro Gly Tyr Val Ala Glu Pro
1810 1815 1820

Gly Pro Pro His Cys Ala Ala Lys Glu
1825 1830

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3759 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..3759

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG CGC CAG GCC GGC GGA TTG GGG CTG CTG GCA CTA CTC CTG CTG GCG	48
Met Arg Gln Ala Gly Gly Leu Gly Leu Leu Ala Leu Leu Leu Ala	
1 5 10 15	
CTG CTG GGC CCC GGC GGC CGA GGG GTG GGC CGG CCG GGC AGC GGG GCA	96
Leu Leu Gly Pro Gly Gly Arg Gly Val Gly Arg Pro Gly Ser Gly Ala	
20 25 30	
CAG GCG GGG GCG GGG CGC TGG GCC CAA CGC TTC AAG GTG GTC TTT GCG	144
Gln Ala Gly Ala Gly Arg Trp Ala Gln Arg Phe Lys Val Val Phe Ala	
35 40 45	

CCT	GTG	ATC	TGC	AAG	CGG	ACC	TGT	CTG	AAG	GGC	CAG	TGT	CGG	GAC	AGC	192
Pro	Val	Ile	Cys	Lys	Arg	Thr	Cys	Leu	Lys	Gly	Gln	Cys	Arg	Asp	Ser	
50						55					60					
TGT	CAG	CAG	GGC	TCC	AAC	ATG	ACG	CTC	ATC	GGA	GAG	AAC	GGC	CAC	AGC	240
Cys	Gln	Gln	Gly	Ser	Asn	Met	Thr	Leu	Ile	Gly	Glu	Asn	Gly	His	Ser	
65					70					75					80	
ACC	GAC	ACG	CTC	ACC	GGT	TCT	GCC	TTC	CGC	GTG	GTG	GTG	TGC	CCT	CTA	288
Thr	Asp	Thr	Leu	Thr	Gly	Ser	Ala	Phe	Arg	Val	Val	Val	Cys	Pro	Leu	
				85					90					95		
CCC	TGC	ATG	AAC	GGT	GGC	CAG	TGC	TCT	TCC	CGA	AAC	CAG	TGC	CTG	TGT	336
Pro	Cys	Met	Asn	Gly	Gly	Gln	Cys	Ser	Ser	Arg	Asn	Gln	Cys	Leu	Cys	
			100					105					110			
CCC	CCG	GAT	TTC	ACG	GGG	CGC	TTC	TGC	CAG	GTG	CCT	GCT	GCA	GGA	ACC	384
Pro	Pro	Asp	Phe	Thr	Gly	Arg	Phe	Cys	Gln	Val	Pro	Ala	Ala	Gly	Thr	
		115					120					125				
GGA	GCT	GGC	ACC	GGG	AGT	TCA	GGC	CCC	GGC	TGG	CCC	GAC	CGG	GCC	ATG	432
Gly	Ala	Gly	Thr	Gly	Ser	Ser	Gly	Pro	Gly	Trp	Pro	Asp	Arg	Ala	Met	
130						135					140					
TCC	ACA	GGC	CCG	CTG	CCG	CCC	CTT	GCC	CCA	GAA	GGA	GAG	TCT	GTG	GCT	480
Ser	Thr	Gly	Pro	Leu	Pro	Pro	Leu	Ala	Pro	Glu	Gly	Glu	Ser	Val	Ala	
145					150					155					160	
AGC	AAA	CAC	GCC	ATT	TAC	GCG	GTG	CAG	GTG	ATC	GCA	GAT	CCT	CCC	GGG	528
Ser	Lys	His	Ala	Ile	Tyr	Ala	Val	Gln	Val	Ile	Ala	Asp	Pro	Pro	Gly	
				165					170					175		
CCG	GGG	GAG	GGT	CCT	CCT	GCA	CAA	CAT	GCA	GCC	TTC	TTG	GTG	CCC	CTG	576
Pro	Gly	Glu	Gly	Pro	Pro	Ala	Gln	His	Ala	Ala	Phe	Leu	Val	Pro	Leu	
			180					185					190			
GGG	CCA	GGA	CAA	ATC	TCG	GCA	GAA	GTG	CAG	GCT	CCG	CCC	CCC	GTG	GTG	624
Gly	Pro	Gly	Gln	Ile	Ser	Ala	Glu	Val	Gln	Ala	Pro	Pro	Pro	Val	Val	
		195					200					205				
AAC	GTG	CGT	GTC	CAT	CAC	CCT	CCT	GAA	GCT	TCC	GTT	CAG	GTG	CAC	CGC	672
Asn	Val	Arg	Val	His	His	Pro	Pro	Glu	Ala	Ser	Val	Gln	Val	His	Arg	
	210					215					220					
ATC	GAG	GGG	CCG	AAC	GCT	GAA	GGC	CCA	GCC	TCT	TCC	CAG	CAC	TTG	CTG	720
Ile	Glu	Gly	Pro	Asn	Ala	Glu	Gly	Pro	Ala	Ser	Ser	Gln	His	Leu	Leu	
225					230					235					240	
CCG	CAT	CCC	AAG	CCC	CCG	CAC	CCG	AGG	CCA	CCC	ACT	CAA	AAG	CCA	CTG	768
Pro	His	Pro	Lys	Pro	Pro	His	Pro	Arg	Pro	Pro	Thr	Gln	Lys	Pro	Leu	
				245					250</							

CCT Pro	TTG Leu	CCT Pro	GGC Gly	CTT Leu	ACC Thr	AAG Lys	CAG Gln	GAA Glu	GAT Asp	TGC Cys	TGC Cys	GGT Gly	AGC Ser	ATC Ile	GGT Gly	864
275280285																
ACT Thr	GCC Ala	TGG Trp	GGA Gly	CAA Gln	AGC Ser	AAG Lys	TGT Cys	CAC His	AAG Lys	TGC Cys	CCA Pro	CAG Gln	CTT Leu	CAG Gln	TAT Tyr	912
290295300																
ACA Thr	GGG Gly	GTG Val	CAG Gln	AAG Lys	CCT Pro	GTA Val	CCT Pro	GTA Val	CGT Arg	GGG Gly	GAG Glu	GTG Val	GGT Gly	GCT Ala	GAC Asp	960
305310315320																
TGC Cys	CCC Pro	CAG Gln	GGC Gly	TAC Tyr	AAG Lys	AGG Arg	CTC Leu	AAC Asn	AGC Ser	ACC Thr	CAC His	TGC Cys	CAG Gln	GAT Asp	ATC Ile	1008
325330335																
AAC Asn	GAA Glu	TGT Cys	GCG Ala	ATG Met	CCC Pro	GGG Gly	AAT Asn	GTG Val	TGC Cys	CAT His	GGT Gly	GAC Asp	TGC Cys	CTC Leu	AAC Asn	1056
340345350																
AAC Asn	CCT Pro	GGC Gly	TCT Ser	TAT Tyr	CGC Arg	TGT Cys	GTC Val	TGC Cys	CCG Pro	CCC Pro	GGT Gly	CAT His	AGC Ser	TTG Leu	GGT Gly	1104
355360365																
CCC Pro	CTC Leu	GCA Ala	GCA Ala	CAG Gln	TGC Cys	ATT Ile	GCC Ala	GAC Asp	AAA Lys	CCA Pro	GAG Glu	GAG Glu	AAG Lys	AGC Ser	CTG Leu	1152
370375380																
TGT Cys	TTC Phe	CGC Arg	CTT Leu	GTG Val	AGC Ser	ACC Thr	GAA Glu	CAC His	CAG Gln	TGC Cys	CAG Gln	CAC His	CCT Pro	CTG Leu	ACC Thr	1200
385390395400																
ACA Thr	CGC Arg	CTA Leu	ACC Thr	CGC Arg	CAG Gln	CTC Leu	TGC Cys	TGC Cys	TGT Cys	AGT Ser	GTG Val	GGT Gly	AAA Lys	GCC Ala	TGG Trp	1248
405410415																
GGT Gly	GCC Ala	CGG Arg	TGC Cys	CAG Gln	CGC Arg	TGC Cys	CCG Pro	GCA Ala	GAT Asp	GGT Gly	ACA Thr	GCA Ala	GCC Ala	TTC Phe	AAG Lys	1296
420425430																
GAG Glu	ATC Ile	TGC Cys	CCC Pro	GGC Gly	TGG Trp	GAA Glu	AGG Arg	GTA Val	CCA Pro	TAT Tyr	CCT Pro	CAC His	CTC Leu	CCA Pro	CCA Pro	1344
435440445																
GAC Asp	GCT Ala	CAC His	CAT His	CCA Pro	GGG Gly	GGA Gly	AAG Lys	CGA Arg	CTT Leu	CTC Leu	CCT Pro	CTT Leu	CCT Pro	GCA Ala	CCC Pro	1392
450455460																
GAC Asp	GGG Gly	CCA Pro	CCC Pro	AAA Lys	CCC Pro	CAG Gln	CAG Gln	CTT Leu	CCT Pro	GAA Glu	AGC Ser	CCC Pro	AGC Ser	CGA Arg	GCA Ala	1440
465470475480																
CCA Pro	CCC Pro	CTC Leu	GAG Glu	GAC Asp	ACA Thr	GAG Glu	GAA Glu	GAG Glu	AGA Arg	GGA Gly	GTG Val	ACC Thr	ATG Met	GAT Asp	CCA Pro	1488
485490495																
CCA Pro	GTG Val	AGT Ile	GAG Gln	GAG Gln	CGA Arg	TCG Arg	GTG Val	CAG Gln	CAG Gln	AGC Gly	CAC Gly	CCC Gly	ACT Gly	ACC Gly	ACC Gly	1536

Pro	Val	Ser	Glu	Glu	Arg	Ser	Val	Gln	Gln	Ser	His	Pro	Thr	Thr	Thr	
			500					505					510			
ACC	TCA	CCC	CCC	CGG	CCT	TAC	CCA	GAG	CTC	ATC	TCT	CGC	CCC	TCC	CCA	1584
Thr	Ser	Pro	Pro	Arg	Pro	Tyr	Pro	Glu	Leu	Ile	Ser	Arg	Pro	Ser	Pro	
		515					520					525				
CCT	ACC	TTC	CAC	CGG	TTC	CTG	CCA	GAC	TTG	CCC	CCA	TCC	CGA	AGT	GCA	1632
Pro	Thr	Phe	His	Arg	Phe	Leu	Pro	Asp	Leu	Pro	Pro	Ser	Arg	Ser	Ala	
		530				535					540					
GTG	GAG	ATC	GCC	JCC	ACT	CAG	GTC	ACA	GAG	ACC	GAT	GAG	TGC	CGA	TTG	1680
Val	Glu	Ile	Ala	Pro	Thr	Gln	Val	Thr	Glu	Thr	Asp	Glu	Cys	Arg	Leu	
545						550				555					560	
AAC	CAG	AAT	ATC	TGT	GGC	CAT	GGA	CAG	TGT	GTG	CCT	GGC	CCC	TCG	GAT	1728
Asn	Gln	Asn	Ile	Cys	Gly	His	Gly	Gln	Cys	Val	Pro	Gly	Pro	Ser	Asp	
				565					570					575		
TAC	TCC	TGC	CAC	TGC	AAC	GCT	GGC	TAC	CGG	TCA	CAC	CCG	CAG	CAC	CGC	1776
Tyr	Ser	Cys	His	Cys	Asn	Ala	Gly	Tyr	Arg	Ser	His	Pro	Gln	His	Arg	
			580					585					590			
TAC	TGT	GTT	GAT	GTG	AAC	GAG	TGC	GAG	GCA	GAG	CCC	TGC	GGC	CCC	GGG	1824
Tyr	Cys	Val	Asp	Val	Asn	Glu	Cys	Glu	Ala	Glu	Pro	Cys	Gly	Pro	Gly	
		595					600					605				
AAA	GGC	ATC	TGT	ATG	AAC	ACT	GGT	GGC	TCC	TAC	AAT	TGT	CAC	TGC	AAC	1872
Lys	Gly	Ile	Cys	Met	Asn	Thr	Gly	Gly	Ser	Tyr	Asn	Cys	His	Cys	Asn	
		610				615					620					
CGA	GGC	TAC	CGC	CTC	CAC	GTG	GGT	GCA	GGG	GGC	CGC	TCG	TGC	GTG	GAC	1920
Arg	Gly	Tyr	Arg	Leu	His	Val	Gly	Ala	Gly	Gly	Arg	Ser	Cys	Val	Asp	
625					630					635					640	
CTG	AAC	GAG	TGC	GCC	AAG	CCT	CAC	CTG	TGT	GGG	GAC	GGT	GGC	TTC	TGC	1968
Leu	Asn	Glu	Cys	Ala	Lys	Pro	His	Leu	Cys	Gly	Asp	Gly	Gly	Phe	Cys	
				645					650					655		
ATC	AAC	TTC	CCT	GGT	CAC	TAC	AAA	TGC	AAC	TGC	TAT	CCT	GGC	TAC	CGG	2016
Ile	Asn	Phe	Pro	Gly	His	Tyr	Lys	Cys	Asn	Cys	Tyr	Pro	Gly	Tyr	Arg	
			660					665					670			
CTC	AAG	GCC	TCC	CGA	CCG	CCC	ATT	TGC	GAA	GAC	ATC	GAC	GAG	TGT	CGC	2064
Leu	Lys	Ala	Ser	Arg	Pro	Pro	Ile	Cys	Glu	Asp	Ile	Asp	Glu	Cys	Arg	
		675					680					685				
GAC	CCT	AGC	ACC	TGC	CCT	GAT	GGC	AAA	TGT	GAA	AAC	AAA	CCT	GGC	AGC	2112
Asp	Pro	Ser	Thr	Cys	Pro	Asp	Gly	Lys	Cys	Glu	Asn	Lys	Pro	Gly	Ser	
		690				695					700					
TTC	AAG	TGC	ATC	GCC	TGC	CAG	CCT	GGC	TAC	CGT	AGC	CAG	GGG	GGC	GGG	2160
Phe	Lys	Cys	Ile	Ala	Cys	Gln	Pro	Gly	Tyr	Arg	Ser	Gln	Gly	Gly	Gly	
705					710					715					720	
GCC	TGT	CGT	GAT	GTC	AAC	GAA	TGC	TCC	GAG	GGT	ACC	CCC	TGC	TCT	CCT	2208
Ala	Cys	Arg	Asp	Val	Asn	Glu	Cys	Ser	Glu	Gly	Thr	Pro	Cys	Ser	Pro	

725	730	735	
GGA TGG TGT GAG AAC CTT CCG GGT TCT TAC CGT TGC ACG TGT GCC CAG Gly Trp Cys Glu Asn Leu Pro Gly Ser Tyr Arg Cys Thr Cys Ala Gln 740 745 750			2256
GGG ATA CGA ACC CGC ACA GGA CGC CTC AGT TGC ATA GAC GTG GAT GAG Gly Ile Arg Thr Arg Thr Gly Arg Leu Ser Cys Ile Asp Val Asp Glu 755 760 765			2304
TGT GAG GCT GGG AAA GTG TGC CAA GAT GGC ATC TGC ACG AAC ACA CCA Cys Glu Ala Gly Lys Val Cys Gln Asp Gly Ile Cys Thr Asn Thr Pro 770 775 780			2352
GGC TCT TTC CAG TGT CAG TGC CTC TCC GGC TAT CAT CTG TCA AGG GAT Gly Ser Phe Gln Cys Gln Cys Leu Ser Gly Tyr His Leu Ser Arg Asp 785 790 795 800			2400
CGG AGC CGC TGT GAG GAC ATT GAT GAA TGT GAC TTC CCT GCG GCC TGC Arg Ser Arg Cys Glu Asp Ile Asp Glu Cys Asp Phe Pro Ala Ala Cys 805 810 815			2448
ATC GGG GGT GAC TGC ATC AAT ACC AAT GGT TCC TAC AGA TGT CTC TGT Ile Gly Gly Asp Cys Ile Asn Thr Asn Gly Ser Tyr Arg Cys Leu Cys 820 825 830			2496
CCC CTG GGT CAT CGG TTG GTG GGC GGC AGG AAG TGC AAG AAA GAT ATA Pro Leu Gly His Arg Leu Val Gly Gly Arg Lys Cys Lys Lys Asp Ile 835 840 845			2544
GAT GAG TGC AGC CAG GAC CCA GGC CTG TGC CTG CCC CAT GCC TGC GAG Asp Glu Cys Ser Gln Asp Pro Gly Leu Cys Leu Pro His Ala Cys Glu 850 855 860			2592
AAC CTC CAG GGC TCC TAT GTC TGT GTC TGT GAT GAG GGT TTC ACA CTC Asn Leu Gln Gly Ser Tyr Val Cys Val Cys Asp Glu Gly Phe Thr Leu 865 870 875 880			2640
ACC CAG GAC CAG CAT GGG TGT GAG GAG GTG GAG CAG CCC CAC CAC AAG Thr Gln Asp Gln His Gly Cys Glu Glu Val Glu Gln Pro His His Lys 885 890 895			2688
AAG GAG TGC TAC CTT AAC TTC GAT GAC ACA GTG TTC TGT GAC AGC GTA Lys Glu Cys Tyr Leu Asn Phe Asp Asp Thr Val Phe Cys Asp Ser Val 900 905 910			2736
TTG GCT ACC AAT GTC ACT CAG CAG GAA TGC TGT TGC TCT CTG GGA GCT Leu Ala Thr Asn Val Thr Gln Gln Glu Cys Cys Cys Ser Leu Gly Ala 915 920 925			2784
GGC TGG GGA GAC CAC TGC GAA ATC TAT CCC TGT CCA GTC TAC AGC TCA Gly Trp Gly Asp His Cys Glu Ile Tyr Pro Cys Pro Val Tyr Ser Ser 930 935 940			2832
GCC GAA TTT CAC AGC CTG GTG CCT GAT GGG AAA AGG CTA CAC TCA GGA Ala Glu Phe His Ser Leu Val Pro Asp Gly Lys Arg Leu His Ser Gly 945 950 955 960			2880

CAA CAA CAT TGT GAA CTA TGC ATT CCT GCC CAC CGT GAC ATC GAC GAA Gln Gln His Cys Glu Leu Cys Ile Pro Ala His Arg Asp Ile Asp Glu 965 970 975	2928
TGC ATA TTG TTT GGG GCA GAG ATC TGC AAG GAG GGC AAG TGT GTG AAC Cys Ile Leu Phe Gly Ala Glu Ile Cys Lys Glu Gly Lys Cys Val Asn 980 985 990	2976
ACG CAG CCC GGC TAC GAG TGC TAC TGC AAG CAG GGC TTC TAC TAC GAT Thr Gln Pro Gly Tyr Glu Cys Tyr Cys Lys Gln Gly Phe Tyr Tyr Asp 995 1000 1005	3024
GGC AAC CTG CTG GAG TGC GTG GAC GTG GAT GAG TGC TTG GAT GAG TCT Gly Asn Leu Leu Glu Cys Val Asp Val Asp Glu Cys Leu Asp Glu Ser 1010 1015 1020	3072
AAC TGC AGG AAC GGA GTG TGT GAG AAC ACA CGT GGC GGC TAC CGC TGT Asn Cys Arg Asn Gly Val Cys Glu Asn Thr Arg Gly Gly Tyr Arg Cys 1025 1030 1035 1040	3120
GCC TGC ACT CCG CCG GCA GAG TAC AGT CCC GCA CAG GCC CAG TGT CTG Ala Cys Thr Pro Pro Ala Glu Tyr Ser Pro Ala Gln Ala Gln Cys Leu 1045 1050 1055	3168
ATC CCG GAG AGA TGG AGC ACG CCC CAG AGA GAC GTG AAG TGT GCT GGG Ile Pro Glu Arg Trp Ser Thr Pro Gln Arg Asp Val Lys Cys Ala Gly 1060 1065 1070	3216
GCC AGC GAG GAG AGG ACG GCA TGT GTA TGG GGC CCC TGG GCG GGA CCT Ala Ser Glu Glu Arg Thr Ala Cys Val Trp Gly Pro Trp Ala Gly Pro 1075 1080 1085	3264
GCC CTC ACT TTT GAT GAC TGC TGC TGC CGC CAG CCG CGG CTG GGT ACC Ala Leu Thr Phe Asp Asp Cys Cys Cys Arg Gln Pro Arg Leu Gly Thr 1090 1095 1100	3312
CAG TGC AGA CCG TGC CCG CCA CGT GGC ACC GGG TCC CAG TGC CCG ACT Gln Cys Arg Pro Cys Pro Pro Arg Gly Thr Gly Ser Gln Cys Pro Thr 1105 1110 1115 1120	3360
TCA CAG AGT GAG AGC AAT TCT TTC TGG GAC ACA AGC CCC CTG CTA CTG Ser Gln Ser Glu Ser Asn Ser Phe Trp Asp Thr Ser Pro Leu Leu Leu 1125 1130 1135	3408
GGG AAG TCT CCG CGA GAC GAA GAC AGC TCA GAG GAG GAT TCA GAT GAG Gly Lys Ser Pro Arg Asp Glu Asp Ser Ser Glu Glu Asp Ser Asp Glu 1140 1145 1150	3456
TGC CGT TGT GTG AGC GGA CGC TGT GTG CCA CGG CCA GGC GGG GCG GTA Cys Arg Cys Val Ser Gly Arg Cys Val Pro Arg Pro Gly Gly Ala Val 1155 1160 1165	3504
TGC GAG TGT CCT GGA GGC TTT CAG CTG GAC GCC TCC CGT GCC CGC TGC Cys Glu Cys Pro Gly Gly Phe Gln Leu Asp Ala Ser Arg Ala Arg Cys 1170 1175 1180	3552

140

Glu Ile Cys Pro Gly Trp Glu Arg Val Pro Tyr Pro His Leu Pro Pro

435	440	445
Asp Ala His His Pro Gly Gly Lys Arg Leu Leu Pro Leu Pro Ala Pro		
450	455	460
Asp Gly Pro Pro Lys Pro Gln Gln Leu Pro Glu Ser Pro Ser Arg Ala		
465	470	475 480
Pro Pro Leu Glu Asp Thr Glu Glu Glu Arg Gly Val Thr Met Asp Pro		
	485	490 495
Pro Val Ser Glu Glu Arg Ser Val Gln Gln Ser His Pro Thr Thr Thr		
	500	505 510
Thr Ser Pro Pro Arg Pro Tyr Pro Glu Leu Ile Ser Arg Pro Ser Pro		
	515	520 525
Pro Thr Phe His Arg Phe Leu Pro Asp Leu Pro Pro Ser Arg Ser Ala		
	530	535 540
Val Glu Ile Ala Pro Thr Gln Val Thr Glu Thr Asp Glu Cys Arg Leu		
545	550	555 560
Asn Gln Asn Ile Cys Gly His Gly Gln Cys Val Pro Gly Pro Ser Asp		
	565	570 575
Tyr Ser Cys His Cys Asn Ala Gly Tyr Arg Ser His Pro Gln His Arg		
	580	585 590
Tyr Cys Val Asp Val Asn Glu Cys Glu Ala Glu Pro Cys Gly Pro Gly		
	595	600 605
Lys Gly Ile Cys Met Asn Thr Gly Gly Ser Tyr Asn Cys His Cys Asn		
	610	615 620
Arg Gly Tyr Arg Leu His Val Gly Ala Gly Gly Arg Ser Cys Val Asp		
625	630	635 640
Leu Asn Glu Cys Ala Lys Pro His Leu Cys Gly Asp Gly Gly Phe Cys		
	645	650 655
Ile Asn Phe Pro Gly His Tyr Lys Cys Asn Cys Tyr Pro Gly Tyr Arg		
	660	665 670
Leu Lys Ala Ser Arg Pro Pro Ile Cys Glu Asp Ile Asp Glu Cys Arg		
	675	680 685
Asp Pro Ser Thr Cys Pro Asp Gly Lys Cys Glu Asn Lys Pro Gly Ser		
	690	695 700
Phe Lys Cys Ile Ala Cys Gln Pro Gly Tyr Arg Ser Gln Gly Gly Gly		
705	710	715 720
Ala Cys Arg Asp Val Asn Glu Cys Ser Glu Gly Thr Pro Cys Ser Pro		
	725	730 735
Gly Trp Cys Glu Asn Leu Pro Gly Ser Tyr Arg Cys Thr Cys Ala Gln		

750

Ala Cys Thr Pro Pro Ala Glu Tyr Ser Pro Ala Gln Ala Gln Cys Leu

[illegible]

1045	1050	1055
Ile Pro Glu Arg Trp Ser Thr Pro Gln Arg Asp Val Lys Cys Ala Gly 1060	1065	1070
Ala Ser Glu Glu Arg Thr Ala Cys Val Trp Gly Pro Trp Ala Gly Pro 1075	1080	1085
Ala Leu Thr Phe Asp Asp Cys Cys Cys Arg Gln Pro Arg Leu Gly Thr 1090	1095	1100
Gln Cys Arg Pro Cys Pro Pro Arg Gly Thr Gly Ser Gln Cys Pro Thr 1105	1110	1115
Ser Gln Ser Glu Ser Asn Ser Phe Trp Asp Thr Ser Pro Leu Leu Leu 1125	1130	1135
Gly Lys Ser Pro Arg Asp Glu Asp Ser Ser Glu Glu Asp Ser Asp Glu 1140	1145	1150
Cys Arg Cys Val Ser Gly Arg Cys Val Pro Arg Pro Gly Gly Ala Val 1155	1160	1165
Cys Glu Cys Pro Gly Gly Phe Gln Leu Asp Ala Ser Arg Ala Arg Cys 1170	1175	1180
Val Asp Ile Asp Glu Cys Arg Glu Leu Asn Gln Arg Gly Leu Leu Cys 1185	1190	1195
Lys Ser Glu Arg Cys Val Asn Thr Ser Gly Ser Phe Arg Cys Val Cys 1205	1210	1215
Lys Ala Gly Phe Thr Arg Ser Arg Pro His Gly Pro Ala Cys Leu Ser 1220	1225	1230
Ala Ala Ala Asp Asp Ala Ala Ile Ala His Thr Ser Val Ile Asp His 1235	1240	1245
Arg Gly Tyr Phe His 1250		

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

Gly Glu Ser Val Ala Ser Lys His Ala Ile Tyr Ala Val Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

[illegible]

Gly Pro Pro Gly Pro Gln Gly Ala Thr Gly Pro Leu Gly Pro Lys Gly
1 5 10 15
Gln Thr Gly Glu Pro Gly Ile Ala Gly Phe Lys Gly Glu Gln Gly Pro
20 25 30
Lys Gly Glu Thr Gly Pro Ala Gly Pro Gln Gly Ala Pro Gly Pro Ala
35 40 45
Gly Glu Glu Gly Lys
50

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 159 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

GGCCCTCCCG	GTCCTCAAGG	TGCAACTGGT	CCTCTGGGCC	CCAAAGGTCA	GACGGGTGAG	60
CCCGGCATCG	CTGGCTTCAA	AGGTGAACAA	GGCCCCAAGG	GAGAGACTGG	ACCTGCTGGG	120
CCCCAGGGAG	CCCCTGGCCC	TGCTGGTGAA	GAAGGAAAA			159

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(A) NAME/KEY: modified_base
(B) LOCATION: 16
(D) OTHER INFORMATION: /note= "N = A or G or C or T"

AAACGTCACA CGTGANACGT GAACGTTGCT TGCTGG 36

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 32 base pairs
 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTACGTCCAC GTACACGTCT AGCAAGCAAG CA

32

002790"5392550